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SOME ASPECTS OF THE DYNAMICS OF THE GLYCOPROTEIN GONADOTROPIN IN GOLDFISH, *CARASSIUS AURATUS*

by

© ALAN FRANK COOK

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Some aspects of the dynamics of the glycoprotein gonadotropin in goldfish, *Carassius auratus*," submitted by Alan Frank Cook in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The present study investigated the clearance and distribution of carp glycoprotein gonadotropin (cGtH) in the goldfish, *Carassius auratus*, during different phases of the annual reproductive cycle. A comparison of injection sites suggested that the intraperitoneal administration of cGtH is inappropriate for hormone clearance studies. The plasma disappearance of intraarterially injected radioiodinated cGtH (^{125}I-cGtH) was found to be multiexponential, exhibiting both a rapid and slow component. The immunoprecipitation of plasma ^{125}I-cGtH employing antisera made against cGtH, provided a significant discrimination between total and trichloroacetic acid (TCA)-precipitated radioactivity.

At 12 ± 1° C, overall plasma metabolic clearance rate (MCR) increased with increasing state of ovarian development. Overall MCR also increases with increasing temperature in sexually regressed goldfish (GSI = 1.4%) but not in sexually maturing fish (GSI = 4.9%) indicating that both temperature and the state of ovarian development can affect the clearance of GtH. Sexually mature female goldfish (GSI = 15.4%) maintained at 12 ± 1° C showed a marked increase in primary MCR and volume of distribution (V_i) compared to all other groups. Plasma GtH levels are elevated in both 20 ± 1° C acclimated regressed and maturing female goldfish, compared to fish maintained at 12 ± 1° C, and at both temperatures the plasma GtH levels increase with increasing GSI. *In vivo* studies

of the uptake of ^{125}I-cGtH by the gonads also indicates a marked influence of both state of ovarian development and temperature. Taken together, these and other data reported in the present study suggest that incremental circulating GtH levels are a reflection of progressive increases in pituitary secretion rate (SR), and are of importance in stimulating progressive development of the gonads.

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INTRODUCTION

To elucidate the mechanisms regulating teleost reproduction, a thorough understanding of gonadotropin (GtH) biochemistry and physiology is essential. GtH's of high specific activity have been prepared from only six teleost species (for review see: Donaldson, 1973; Fontaine, 1976; Peter and Crim, 1979). The number of GtH's elaborated by the teleost pituitary gland is currently a subject of controversy. Salmon GtH, SG G-100 (Donaldson *et al.*, 1972), although prepared from pituitary glands of sexually mature *Oncorhynchus tshawytscha*, is capable of stimulating all stages of sexual development (Donaldson, 1973; Sundararaj and Anand, 1972; Sundararaj *et al.*, 1972a; Fontaine, 1976). Whether or not SG G-100 contains more than one GtH has not been determined. However, Pierce *et al.* (1976) demonstrated a considerable amount of material, in addition to the classical glycoprotein GtH, in Donaldson's SG G-100 that lacks affinity to concanavalin A (Con A)-sepharose. Highly purified carp GtH (cGtH) (Burzawa-Gerard, 1969, 1971, 1974; Jolles *et al.*, 1977) is also capable of stimulating a number of stages in the sexual development of teleost fishes (Fontaine, 1975). Billard *et al.* (1970) used 1 µg cGtH per g body weight (BWt) to restore all aspects of spermatogenesis and spermiation in hypophysectomized male goldfish, *Carassius auratus*. Carp GtH is also capable of stimulating vitellogenesis, ovarian maintenance and ovulation in hypophysectomized catfish, *Heteropneustes fossilis* (Sundararaj *et al.*, 1972b). More recently

Sundararaj *et al.* (1976) reported the maintenance of vitellogenesis in hypophysectomized *H. fossilis* with very low doses of cGtH. Since the fish were gravid at the beginning of the experiment and because hormone injections began the day after hypophysectomy, it is not possible to determine whether or not the cGtH was capable of stimulating the incorporation of yolk into the oocyte, or rather just maintaining the existing mature oocytes. The study of Burzawa-Gerard (1974) is the only additional published evidence supporting a role of cGtH in vitellogenesis. However, because of the high dosage (5 µg cGtH per g BWt) used to reinitiate vitellogenesis in hypophysectomized female *C. auratus* and since only one of the injected fish showed a gonosomatic index (GSI) (see METHODS AND MATERIALS) similar to intact fish, these results are difficult to interpret. Evidence for two GtH's of distinct physico-chemical and biological characteristics in chum salmon (Ng and Idler, 1978a) and plaice (Ng and Idler, 1978b) is unequivocal. Idler and associates have purified a vitellogenic GtH characterized by a lack of affinity to Con A-sepharose (Campbell and Idler, 1976; Ng and Idler, 1978a,b). This nonglycoprotein GtH is capable of stimulating yolk incorporation into the ovary (Campbell, 1978), but after rechromatography on Con A-sepharose is incapable of inducing final maturation and ovulation in hypophysectomized test fish (Campbell and Idler, 1977; Ng and Idler, 1978a,b). The glycoprotein GtH, although very potent in oocyte maturation and ovulatory activity, is devoid of vitellogenin uptake activity (Campbell and Idler, 1976; Campbell, 1978). A highly

purified glycoprotein salmon GtH prepared from pituitary glands of rainbow trout, *Salmo gairdneri* (Breton *et al.*, 1978), is capable of stimulating the synthesis of endogenous yolk in the ooplasm of juvenile rainbow trout ovaries (Upadhyay, 1977). The incorporation of vitellogenin synthesized in the liver under the influence of estrogens was stimulated not by the glycoprotein GtH but rather by a factor(s) in the crude salmon pituitary extract (Upadhyay, 1978). Whether or not this pituitary factor(s) is a nonglycoprotein GtH has not been determined.

The effects of environmental factors on serum or plasma GtH levels provide insight into the mechanisms whereby fish time their reproductive cycles with environmental cues (for reviews see: de Vlaming, 1974; Peter and Hontela, 1978; Peter and Crim, 1979). The majority of studies demonstrate that warm temperatures are associated with increased blood GtH levels (Gillet *et al.*, 1977; Gillet *et al.*, 1978; Breton and Billard, 1977; Breton *et al.*, 1975; Hontela and Peter, 1978) and suggest that elevated circulating GtH titres can either accelerate gonadal recrudescence or regression (Gillet *et al.*, 1978) depending on condition of the gonad and time of year. Hontela and Peter (1978) described daily variations in serum GtH levels in female goldfish at three different times of the year, under different combinations of temperature and photoperiod. These authors suggest that the mechanism for stimulation of gonadal recrudescence under long photoperiod and warm temperature is by surges of GtH secretion during each 24 hr light dark cycle (Hontela and Peter, 1978) as opposed to alterations in the basal

GtH levels as previously supposed (Gillet *et al.*, 1977). Elevated serum or plasma GtH levels are established in the endocrine control of spermiation (Breton *et al.*, 1972; Crim and Evans, 1978; Sanchez-Rodriguez *et al.*, 1978) and ovulation (Crim *et al.*, 1973, 1975; Breton *et al.*, 1975; Fostier *et al.*, 1978; Jalabert *et al.*, 1978; Stacey *et al.*, 1979a,b) in teleost fishes. It is clear that studies of plasma GtH levels have increased our understanding of the mechanisms regulating teleost reproduction. To date, however, no method exists for the measurement of circulating nonglycoprotein GtH in teleost fishes. The uncertainty regarding the existence of this hormone in a number of species, (e.g. carp, see above), means some of the hypotheses derived on the basis of the above data must be regarded as tentative until this issue is resolved for each species under study. Existing teleost GtH radioimmunoassays (RIA) developed using highly purified GtH's (carp, goldfish - Breton *et al.*, 1971; Crim *et al.*, 1976; Hontela and Peter, 1978; rainbow trout - Breton and Billard, 1977) probably measure only the glycoprotein GtH provided a glycoprotein tracer is employed (see METHODS AND MATERIALS). RIA's based on antibodies raised against an antigen preparation which may contain appreciable amounts of more than one distinct GtH (Crim *et al.*, 1975) must be viewed with caution. In addition, studies of static GtH concentrations in serum or plasma, and the pituitary gland indicate little about the dynamic physiology of this hormone.

Although many studies correlate plasma GtH levels with a particular experimental treatment or physiological event, assessment

of the GtH dynamics requires evaluation of all the relevant parameters: the plasma metabolic clearance rate (MCR), pituitary secretion rate (SR), and plasma and pituitary GtH concentrations. Recent studies concerning the role of GtH in the precocious sexual development of male Atlantic salmon (*Salmo salar*) (Dodd *et al.*, 1978; Crim and Evans, 1978), illustrate the necessity of assessing GtH dynamics. Crim and Evans (1978) hypothesize that the depletion of pituitary GtH in July and August may be related to an increase in hormone release as this period coincides with the onset of precocious gonad development and an increase in plasma GtH titres. In addition these authors speculate that the observed elevated GtH levels in both pituitary and plasma in fall and winter, as well as the declining hormone levels observed from March to June (Dodd *et al.*, 1978; Crim and Evans, 1978), represent a period of GtH release which is important for germ cell mitotic division. However, alternative explanations are possible and cannot be ruled out without quantitative information on the clearance and distribution of this hormone. For example, both an increase in testicular binding of GtH (see Channing and Kammerman, 1974; Catt *et al.*, 1973) and a decrease in water temperature (Crim and Evans, 1978) would cause a decrease in the MCR of GtH during the winter period of spawning and spermiation. A decreased MCR could be responsible for the observed elevated plasma levels during the spawning season; in addition plasma GtH could have feedback actions on the hypothalamus and/or pituitary to inhibit further release of pituitary GtH and, unless synthesis decreased significantly, pituitary GtH content would be elevated as

observed. Perhaps in the spring, as water temperature and GtH MCR increases, plasma GtH levels decline removing the pituitary inhibition, facilitating the observed decline in pituitary GtH content (Crim and Evans, 1978). In these and other studies where GtH has been measured, information concerning the GtH dynamics would obviously facilitate the interpretation of results.

There is only limited information available concerning the clearance of teleost GtH from the plasma with time (plasma disappearance profiles) after a single injection (Crim and Evans, 1976). In their study, immature rainbow trout (*S. gairdneri*) were injected intraperitoneally (ip) with either 0.02 or 0.2 µg GtH/g body weight, at three different temperatures. Blood samples were collected at 1, 2, 3 and 7 days after the GtH or vehicle injection. No analysis was made to determine the half-life ($t_{\frac{1}{2}}$) or MCR of the hormone from the plasma. The plasma GtH disappearance profiles measured by RIA decreased in magnitude with decreasing temperature, suggesting that the rate of clearance of GtH from the plasma decreased with decreasing temperature. The profiles indicated that detectable levels of GtH can be found in the blood for up to 1 week following a single injection and that treatment with microgram doses of GtH develops plasma hormone levels in the ng per ml range.

It is the purpose of the present investigation to define the plasma MCR, SR and initial biological half-life ($t_{\frac{1}{2}_i}$) of glycoprotein cGtH in female goldfish acclimated to 12 ± 1° C and 20 ± 1° C, at three separate stages in the annual reproductive cycle. In addition a comparison to regressed male goldfish will be

made at 20 ± 1° C. The GtH MCR will be determined using the single injection method measuring the disappearance of total, trichloroacetic acid (TCA)-precipitated and immunoprecipitated radioiodinated cGtH. A comparison of injection sites will be made to determine which is most appropriate for hormone clearance studies. Plasma GtH concentrations will be assayed by RIA, and by use of MCR data, the pituitary secretion rate will be estimated.

METHODS AND MATERIALS

Source and Maintenance of Fish

Goldfish, *Carassius auratus*, of the common or comet varieties were purchased from Grassyfork Fisheries Ltd., Martinsville, Indiana. All fish were maintained for a minimum of 28 days in 1500 l flow-through aquaria under a simulated natural photoperiod (Edmonton, Alberta, Canada) prior to experimental treatment. During this period the water temperature was 20 ± 1° C prior to commencing experiments with sexually regressed goldfish and 12 ± 1° C for sexually maturing and mature fish. At random times during the daily photophase the fish were fed a commercial trout chow (Ewos) *ad libitum*.

Part I: Intraperitoneal Injections of cGtH

In spite of the theoretical advantages of excluding the pituitary gland as a source of endogenous GtH production for assessing the disappearance characteristics of the exogenous carp gonadotropin (cGtH) by the single injection technique, this approach was rejected in view of the pronounced hemodynamic (A.F. Cook, personal observation) and metabolic (Walker and Johanson, 1977) changes associated with goldfish hypophysectomy. A possible source of error with intact fish is the nonspecific stress component of the intraperitoneal injection of cGtH; to obviate this possibility the effect of the injection *per se*

was determined by including a number of control groups. After the initial acclimation period (see above) male fish were randomly divided into three groups, and reacclimated in 75 l plastic aquaria maintained at 12 ± 1° C under a light dark cycle consisting of 12 hr light alternating with 12 hr dark (lights on at 0800 hr). The fish were fed Tetramin at 0900 hr daily. Prior to injection or blood sampling the fish were anaesthetized by immersion in 0.05% tricaine methanesulfonate (TMS) (Sigma), buffered to pH 7.0 with 0.5 M imidazole (modified from Strange and Schreck,1978), until all visible muscular movement ceased. After 10 days, all fish in group I were bled (pre-experiment sample) by the standard method (see below) between 1400 and 1500 hr; the remaining fish were undisturbed at this time. After 7 days recovery, group I fish received intraperitoneal injections of 0.025 µg cGtH (Burzawa-Gerard, 1971) per g body weight (BWt) (5 µg cGtH per ml teleost saline) with a 250 µl Hamilton syringe fitted with a disposable 27 gauge x ½" needle. Blood samples were taken at either 1, 3, 6, 12, 18, 24, 72 or 168 hr after injection. All samples were taken at the same time in the daily photophase as the presample (1400 - 1500 hr), thus each fish in this group served as its own control. Vehicle-injected group II fish received an equivalent volume of teleost saline and were sampled identical to group I. The uninjected control group (group III) was sampled on the same schedule as groups I and II. Blood samples were processed for subsequent GtH RIA as described below. No fish were sampled more than twice after injection or within a 48 hr period. Since there were no significant differences

in the GtH values between the various control groups in this experiment (see RESULTS), the values obtained from the self-control group (pre-experiment sample) were used as a measure of endogenous serum GtH levels in subsequent experiments. Two additional experiments were done, one using male fish at 20 ± 1° C and the other using female fish at 12 ± 1° C, with the same protocol as described above, except that groups II and III were not included. To dissociate the disappearance pattern of the exogenous hormone from that of the total (endogenous and exogenous) GtH, the individual GtH values obtained from the pre-experimental sample were subtracted from the values obtained after the intraperitoneal injection of cGtH. The disappearance of exogenous GtH after the intraperitoneal injection, when expressed as a per cent of the administered dose per ml serum, exhibited a linear disappearance pattern on a semi-logarithmic plot (Figure 5) evidencing first-order kinetics in a single compartment (Shipley and Clark, 1972). The disappearance profile may be described by the equation:

$$x'(t) = A\, e^{-\alpha t} \qquad (1)$$

where the concentration of exogenous hormone at any time after the intraperitoneal injection, $x'(t)$, is a function of the ordinate intercept A and the slope α. The serum MCR and biological half-disappearance time ($t_{\frac{1}{2}}$) were calculated as described by Tait and Burstein (1964) and Gurpide (1975), as described in APPENDICES I and II. Since secretion must balance metabolism under steady-state conditions (Gurpide, 1975), the pituitary secretion rate (SR) is

equivalent to the product of the MCR and the circulating (endogenous) GtH concentration.

Part II: Ovarian Uptake of ^{125}I-cGtH and ^{125}I-BSA by Female Goldfish Acclimated to 20 ± 1° C

Radioiodination of carp gonadotropin (^{125}I-cGtH) and bovine serum albumin (^{125}I-BSA) (Sigma) was accomplished by similar methods, as described below (*Preparation of radioiodinated gonadotropin and bovine serum albumin*).

Each fish received approximately 7 ng of ^{125}I-cGtH (about 3.9×10^6 cpm per fish) via intraarterial injection (see below). Teleost saline (Burnstock, 1958) was employed as a diluent to make a total injection volume of 50 µl. The fish were sacrificed at timed intervals (20 to 60 minutes) following injection, as indicated in Figure 5. Immediately after sacrifice, the ovaries were removed, dissected free of adipose tissue and individually weighed. A minimum of 5 fish were used for each sample, except where noted (Figure 6). The radioactivity of each ovary was counted (Model MS 588 Micromedic Systems Inc. Ltd., Horsham, PA.) and the results were expressed as per cent of injected dose per g ovary. Portions of other tissues, including muscle and intestine were also weighed, and their radioactivity measured. Aliquots of plasma were also counted for radioactivity. The same experiment was repeated substituting ^{125}I-BSA for ^{125}I-cGtH as the injected tracer.

Competitive uptake experiments

Groups of female fish acclimated to 20 ± 1° C (12L:12D; lights on at 0800 hr) received intraperitoneal injections at 0800 hr of either 0.50 µg BSA per g BWt, 0.35 µg cGtH per g BWt, or 0.50 µg cGtH per g BWt. The unlabelled hormone and BSA were each dissolved in teleost saline such that each fish received 5 µl of solution per g BWt. Three hr later each fish received about 7 ng freshly iodinated ^{125}I-cGtH (or about 3.9 x 10^6 cpm) via intraarterial injection (see below). All fish which received intraperitoneal injections of unlabelled cGtH were sacrificed 30 minutes after the intraarterial injection of ^{125}I-cGtH, and the ovaries were removed, weighed and their radioactivity measured as described. The fish pre-injected with BSA were sacrificed at the timed intervals (20 to 60 minutes) following the intraarterial injection, as indicated in Figure 6.

Part III: Clearance of Intraarterially Injected Glycoprotein ^{125}I-cGtH

Experimental protocol and sampling procedure

Immature fish (August - September, 1978)

After the initial acclimation period, one group of 50 female goldfish was gradually cooled to 12 ± 1° C over a 24 hr period and reacclimated for 14 days, while a second group of 120 male and female fish was exposed to the initial acclimation conditions for a further 14 days.

Maturing fish (February 1979)

After acclimation to 12 ± 1° C, one group of 50 maturing female goldfish was gradually warmed to 20 ± 1° C over a 24 hr period and reacclimated for 14 days, while the remaining fish were left at 12 ± 1° C.

Mature fish (March 1979)

Mature female goldfish were acclimated for at least 14 days to 12 ± 1° C prior to experimental treatment. Aquatic vegetation was omitted from the aquaria as it is known to be a specific cue for ovulation in the goldfish (Stacey *et al.*, 1979b).

To start the experiment with each of the experimental groups defined above, 48 hr prior to the injection of ^{125}I-cGtH, the fish were anaesthetized and about 150 µl of blood was withdrawn from the caudal vasculature with a 25 gauge x 5/8" needle fitted to a 1 ml disposable syringe. Each syringe was pretreated with about 3 mg of diamino ethanetetra-acetic acid, disodium salt (Na EDTA). The samples were kept on chipped ice for 2 to 3 hr prior to centrifugation at 760 x g for 20 min at 4° C. The plasma was stored at -25° C until GtH RIA analysis. After sampling, the fish were transferred to experimental 75 l aquaria. These aquaria are as described by Stacey *et al.* (1979a) with the addition of plastic liners for radiation containment.

Blood samples (about 300 µl) were taken from individual fish at 10, 15, 20, 30, 55, 75 and 120 min after intraarterial injection of ^{125}I-cGtH (see below for description of ^{125}I-cGtH). Duplicate 25 µl aliquots of plasma were assayed for total radioactivity in an

automatic gamma radiation counter (Model MS 588, Micromedic Systems Inc. Ltd., Horsham, PA.). The total radioactivity determined in the plasma of experimental fish was expressed as a per cent of the injected total radioactivity per ml plasma after standardization to 25 g BWt. The latter calculation assumes the kinetics of ^{125}I-cGtH in goldfish to be linearly related to body weight. Remaining plasma was utilized for trichloroacetic acid (TCA) precipitable and immunoprecipitable assays (see below).

Preparation of radioiodinated gonadotropin and bovine serum albumin

Short-term clearance studies were performed with cGtH iodinated with iodine-125 by use of chloramine T (Greenwood *et al.*, 1963). Unreacted iodide and damaged hormone were separated from intact ^{125}I-cGtH by gel filtration on a Sephadex (Pharmacia, Uppsala, Sweden) G-50 (fine) column (1.1 x 10 cm) using barbital buffer (0.08 M, pH 8.6). The specific activity of labelled cGtH was estimated by RIA, comparing increasing amounts of label with a small quantity of label and increasing amounts of unlabelled cGtH, similar to Bolton (1977), and also by estimating the per cent of protein radioiodination (Bolton, 1977). The two methods yielded similar estimates of specific activity of approximately 190 μCi/μg. One-half ml of the protein peak from the Sephadex G-50 column was applied to a Con A-sepharose (Pharmacia) column (2 cm^3 Con A-sepharose in a 5 ml plastic syringe) and first eluted with barbital buffer to remove the nonglycoprotein ^{125}I-cGtH, followed by 0.15 M 1-O-methyl-α-D-glucopyranoside (Sigma) in barbital buffer to obtain

glycoprotein ^{125}I-cGtH. The glycoprotein ^{125}I-cGtH was diluted with teleost saline such that each 50 µl injection volume contained about 300,000 cpm and an estimated 5 pg GtH. Intraarterial injections of ^{125}I-cGtH were made with a 50 µl Hamilton syringe fitted with a disposable 30 gauge x ½" needle. BSA was radioiodinated and chromatographed on Sephadex G-50 (fine) the same as for cGtH. The ^{125}I-BSA separated by Sephadex chromatography had an estimated specific activity of 175 µCi/µg.

Gonadotropin radioimmunoassay

Details of the GtH RIA have been described (Crim *et al.*, 1976; Hontela and Peter, 1978). The RIA involves a competition between goldfish GtH in the serum or pituitary samples, and glycoprotein ^{125}I-cGtH with a limited quantity of specific rabbit anti-cGtH antisera.

Intraarterial injection procedure

After TMS anaesthesia as described above, the fish were lightly blotted on absorbent paper and weighed (± 0.005 g). The fish were then wrapped in moistened tissue paper and positioned, ventral side up, in a wedge-shaped foam-rubber support. A mid-ventral incision through the hypaxial musculature from the junction of the opercular membranes to the base of the right pectoral fin exposed the pectoral girdle. While viewing under a dissecting microscope, fine-point scissors were used to cut the pectoral girdle, exposing the pericardial cavity, and retractors were used

to spread the pectoral girdle. The injection was then made into the bulbous arteriosus, selected as the injection site because of its thick musculature and volume compensatory function. In most cases the pericardium was teased from the bulbous prior to injection. After injecting glycoprotein ^{125}I-cGtH, the needle was left in position for about 20 heart beats. The incision was filled with physiological solution (Burnstock, 1958) and closed with a single suture of 4-0 surgical thread. In all cases, respiratory and locomotory movements were regained within 8 minutes of injection. Throughout the operative procedure care was taken to prevent any excessive hemorrhage; fish showing arterial hemorrhage were discarded from the experiment. The entire operative procedure was routinely accomplished in less than 5 minutes.

Tissue ^{125}I-cGtH uptake

After the blood sample was taken, the fish was killed by making a V-shaped cut through the spinal column just posterior to the head region and the heart was removed to stop further circulation. All tissues were trimmed of attached adipose tissue, rinsed in saline, blotted on absorbent paper and weighed (± 0.05 mg). The gonosomatic index (GSI) for each fish was calculated as follows: GSI = (gonadal weight/total body weight) x 100. After homogenization for 1 minute in 20 per cent (w/v) TCA (about 10 ml per g tissue) with either a Teflon and ground glass or a Polytron PT-10 homogenizer, the samples were treated in a manner identical to the TCA analysed plasma samples. Muscle samples were taken from the

trunk musculature and hypothalami were dissected as described by Crim *et al.* (1976).

Trichloroacetic acid precipitation

Duplicate 25 µl aliquots of test plasma were mixed with 1 ml of 20 per cent (w/v) TCA in 12 x 75 mm disposable glass test tubes. After vortexing, the samples were allowed to stand on chipped ice for 10 minutes prior to centrifugation at 760 x g for 20 minutes at 4° C. The supernatants were aspirated and discarded and the precipitate washed with 1 ml of 20 per cent TCA, vortexed, and centrifuged as before. The pellet was counted in an automatic gamma radiation counter. TCA precipitable radioactivity injected into each fish was determined for standardization purposes in identical fashion on duplicate 25 µl volumes of ^{125}I-cGtH diluted 1:4 (v/v) with goldfish plasma from a pool obtained from male and female goldfish acclimated to the experimental conditions. The recovery of ^{125}I-cGtH as TCA-precipitated radioactivity added to pool goldfish plasma varied but was never less than 74 per cent. The values of TCA-precipitated radioactivity are expressed as a per cent of the injected dose assayed in identical fashion, per ml plasma after standardization to a 25 g BW.

Immunoprecipitation

Rabbit anti-carp (RAC) antisera* concentrations, ranging from

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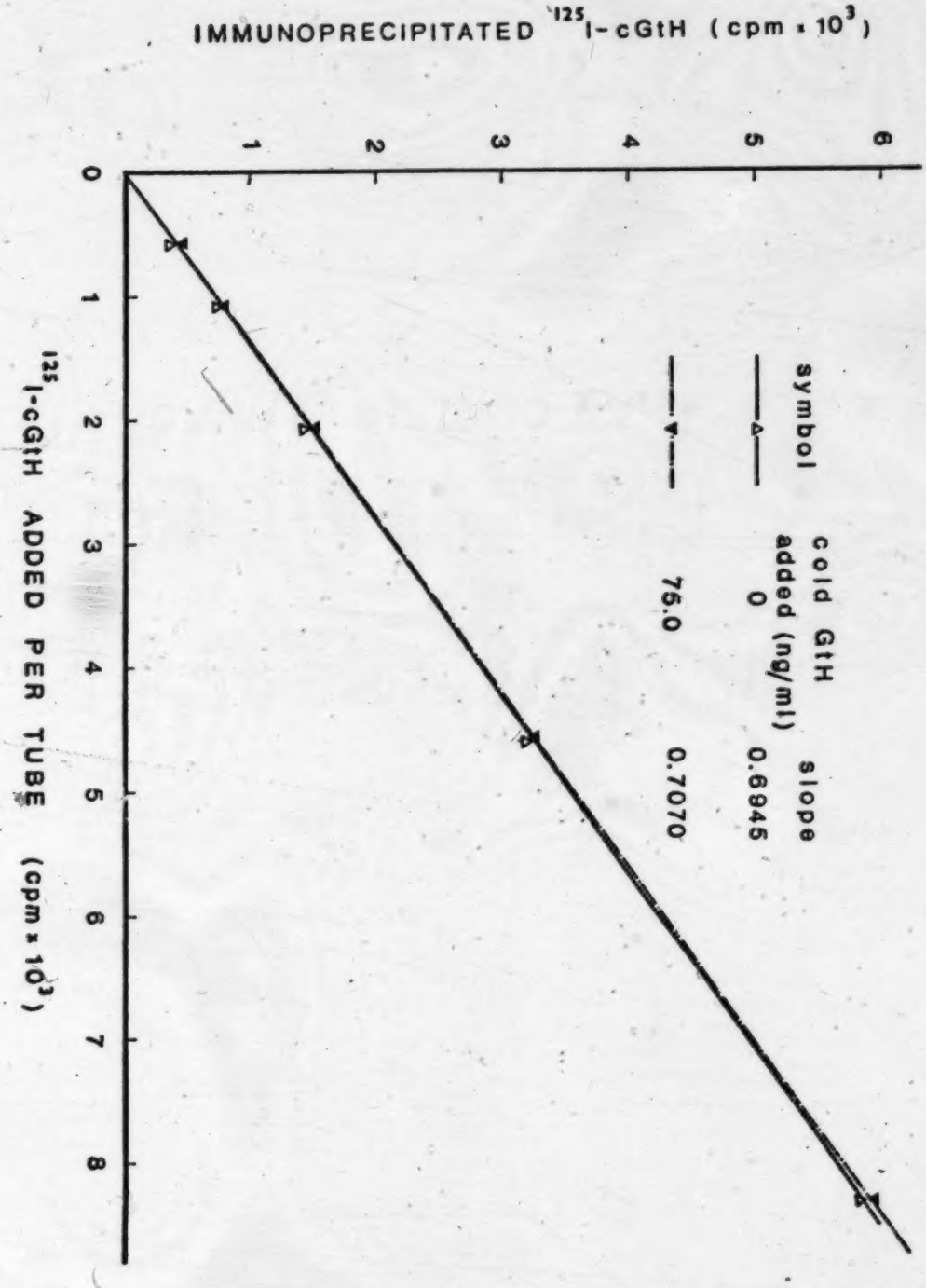
* A gift of Dr. L. Crim, Marine Sciences Research Laboratory, The Memorial University of Newfoundland, St. Johns, Newfoundland.

Fig. 1. Recovery* of immunoprecipitated ^{125}I-cGtH after the addition of either 20 µl of buffer (open triangles) or 20 µl of buffer containing 75 ng cGtH per ml (solid triangles). The ^{125}I-cGtH was also added in a volume of 20 µl. The addition of the unlabelled cGtH does not affect the immunoprecipitation of ^{125}I-cGtH. Values shown are means of duplicate determinations.

* Immunoprecipitation system consisted of 1:14400 x and 1:80 x dilution of rabbit anti-carp gonadotropin antisera and normal rabbit serum, respectively, in 400 µl of barbital buffer antibody precipitation as described by Crim *et al.* (1976).


IMMUNOPRECIPITATED ^{125}I-cGtH (cpm × 10^3)
0
1
2
3
4
5
6
^{125}I-cGtH ADDED PER TUBE (cpm × 10^3)
0
1
2
3
4
5
6
7
8
symbol
cold GtH added (ng/ml)
slope
0
0.6945
76.0
0.7070

1:210 to 1:73500 dilutions** in sodium barbital buffer were tested for their ability to precipitate varying amounts of ^{125}I-cGtH diluted to 20 µl with hypophysectomized goldfish plasma. The second antibody system employed in these tests was the same as in the GtH RIA (Crim *et al.*, 1976). A 1:2000 dilution of RAC antisera produced a binding maxima to which the second antibody precipitation system was them optimized. The selected RAC antiserum and normal rabbit serum (NRS) concentrations, 1:2000 x and 1:80 x respectively, were diluted in 400 µl of assay buffer, dispensed into 12 x 75 mm disposable glass test tubes, frozen over dry ice and stored at -25° C until use. The goat anti-rabbit (GAR) antisera was used at a 1:420 dilution in 100 µl of assay buffer. Incubation times and temperatures, and centrifugation procedures were as described for the GtH RIA (Crim *et al.*, 1976).

In addition, tests were carried out to demonstrate non-interference of endogenous GtH, at physiological concentrations, in the measurement of ^{125}I-cGtH in goldfish plasma samples. Concentrations from 2.5 to 75.0 ng carp GtH per ml of pooled plasma were assayed in volumes of 20 µl with the immunoprecipitation system above, using a wide range of ^{125}I-cGtH concentrations (Figure 1).

To measure ^{125}I-cGtH in plasma from experimental animals, a single 20 µl volume was immunoprecipitated using the system as described above. The limited supply of the RAC antisera precluded

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** Many investigators have stated antisera titres as dilutions added to the assay tube. Herein, all antisera dilutions represent the final concentration in the assay tube.

the possibility of duplicate determinations. As with the TCA-precipitated radioactivity determinations, 20 µl of injection volume was assayed in an identical manner after dilution with a plasma pool, for standardization purposes. The specific binding varied between 74 and 83 per cent. The values of immunoprecipitated radioactivity are expressed as a per cent of the injected dose assayed in identical fashion, per ml plasma after standardization to a 25 g BWt.

Data analysis and statistics

The data of the disappearance curves or metabolic clearance profiles (MCP), expressed as a per cent of administered dose per ml plasma, was curvilinear when plotted against time (t) on semi-logarithmic paper. Each MCP was resolved into the sum of two exponential functions in the following manner. Initially each MCP was analysed by the method of curve peeling (Shipley and Clark, 1972). With this method, a straight line of slope β and ordinate intercept B is fitted by the method of least squares (Zar, 1974) through the terminal portion of each curve between 30 and 120 minutes. The values at 10, 15, 20 and 30 minutes on the above line extrapolated to t = 0 are subtracted from the initial part of the MCP between 10 and 30 minutes. A second line is then fitted by least-squares through these derived points with slope α and ordinate intercept A (Figure 11). The curve represented by these straight lines is:

$$x'(t) = A\, e^{-\alpha t} + B\, e^{-\beta t} \qquad (2)$$

where x'(t) is the radioactive concentration expressed as a per cent of injected dose per ml plasma at time t. The values of the parameters A, α, B and β were then used as starting points in a computerized non-linear least squares method BMD-07R (Dixon, 1974). With this program an error mean square (EMS) is computed for the equation x'(t) with the user-supplied initial parameters and the experimental data. The parameters are then altered simultaneously, minimizing the EMS to a user-specified convergence criterion. In addition to removing subjective errors of bias on the part of the experimenter, this method provides advantages over 'curve-peeling' as the parameters are equally well-determined and relevant parameter error estimates are obtained. The validity of the fit of each MCP was estimated by the per cent variance accounted for and standard error of estimate (Zar, 1974).

Computation of plasma metabolic clearance rate, initial half-life and volume of distribution

A variety of computational methods were employed to determine the most reliable and error-resistant estimate of the overall MCR (see APPENDIX II). The area beneath each MCP was integrated analytically from t = 0 to t = 120 minutes (Campbell *et al.*, 1978a). Integration using a numerical method (Normand and Fortier, 1970) gave values of MCR in excellent agreement with the analytical method. An estimate of the 'primary' MCR (Campbell *et al.*, 1978a) was also evaluated.

The values of initial half-life ($t_{\frac{1}{2}_i}$) (see APPENDIX I) and distribution volume of the first compartment (V_i) were computed using the formulae of Shipley and Clark (1972) and Wagner and Northam (1967), respectively.

Statistics

A single value for each of MCR, $t_{\frac{1}{2}i}$ and V_i was computed from each MCP. Since it was not possible to repeat individual experiments a number of times the evaluation of the error terms for these values was determined using the general formula derived in APPENDIX III. Statistical differences between groups were determined by analysis of variance and the Duncan multiple range test or the Students' t-test where applicable (Steel and Torrie, 1960). A p value of 0.05 or less was considered statistically significant.

RESULTS

Part I: Intraperitoneal Injections of cGtH

The results of the experiment using sexually mature male goldfish (GSI = 3.41 ± 0.09%) maintained at 12 ± 1° C under a 12L:12D (lights on at 0800 hr) light dark cycle are shown in Figure 2. Since the serum GtH levels did not differ significantly (Duncan's multiple range test) either within each of the control groups or between the control groups at any sample time (mean value 3.14 ± 0.16 ng/ml), the uninjected and vehicle-injected control groups were omitted from subsequent experiments. Fish receiving the cGtH had significantly elevated ($p < 0.01$) serum GtH levels compared to the control values at the first sample after injection (1 hr), and the maximum level (44.67 ± 3.72 ng/ml) was obtained 3 hr post-injection. Serum GtH remained significantly greater than the control values until the 48 hr post-injection sample; there were no significant differences in the serum GtH levels between the fish receiving the cGtH and the self-control values for the remainder of the sampling period (Figure 2).

Figure 3 illustrates the serum GtH levels in sexually mature male goldfish (GSI = 2.63 ± 0.23%) maintained under 12L:12D/20±1° C. No significant differences in serum GtH levels were detected between sample times for the self-control values (mean value 11.63 ± 1.02 ng/ml). Following injection of cGtH, the maximum GtH level (67.91 ± 3.12 ng/ml) was obtained at 1 hr post-injection. The serum

Fig. 2. Serum GtH profiles in sexually mature male goldfish at 12 ± 1° C. The 'self-control' group (open stars) was sampled 7 days prior to intraperitoneal injection of 0.025 µg cGtH per g body weight (so]id squares) at the same time of day as the post-injection sample. The vehicle-injected control group (open squares) received 5 µl of teleost saline per g body weight. The uninjected control group is represented by the solid stars. There was no difference in GSI between groups (pooled GSI = 3.41 ± 0.09%). Values are mean ± SE (N = 8 to 14).

Serum Disappearance Profile of Immunoreactive cGtH After a Single Intraperitoneal Injection in Sexually Mature Male Goldfish Maintained at 12°C. GSI = 3.41 ± 0.09 %

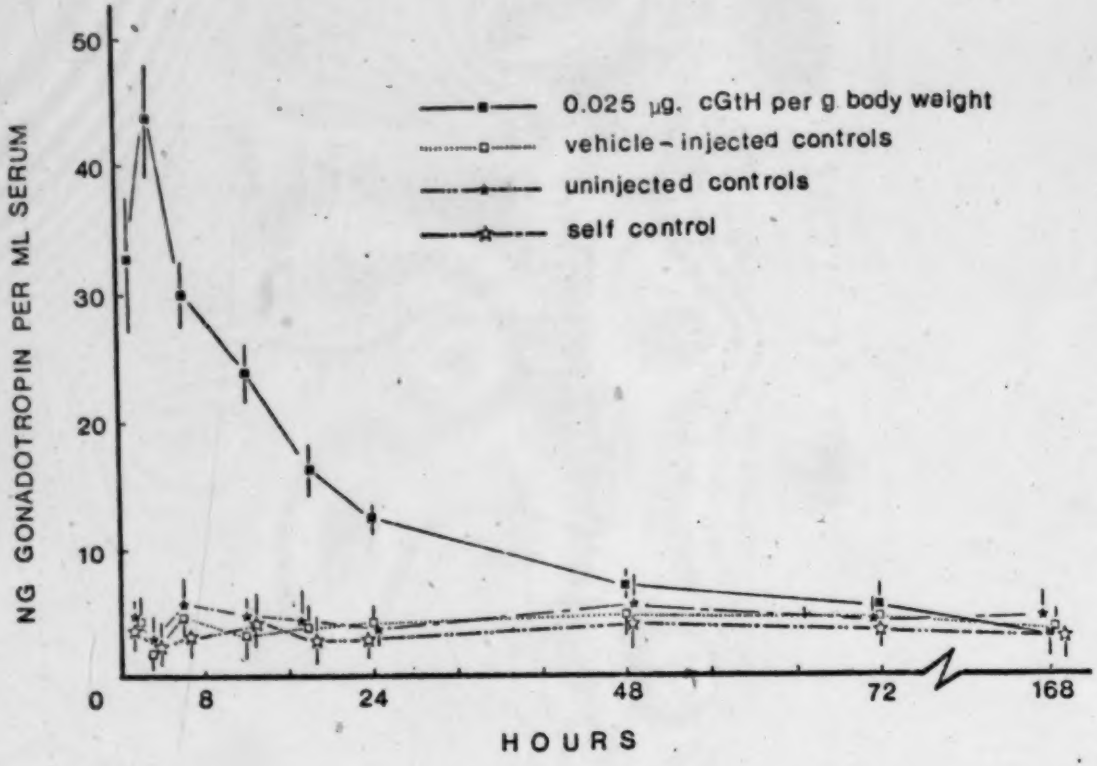


Fig. 3. Serum GtH profiles in sexually mature male goldfish at 20 ± 1° C. The 'self-control' group (solid stars) was sampled 7 days prior to a single intraperitoneal injection of 0.025 µg cGtH per g body weight (solid squares). There was no difference in GSI between groups (pooled GSI = 2.63 ± 0.23%). Values are mean ± SE (N = 8).

Serum Disappearance Profile of Immunoreactive cGtH After a Single Intraperitoneal Injection in Sexually Mature Male Goldfish Maintained at 20°C. G S.I. = 2.63 ± 0.23 %

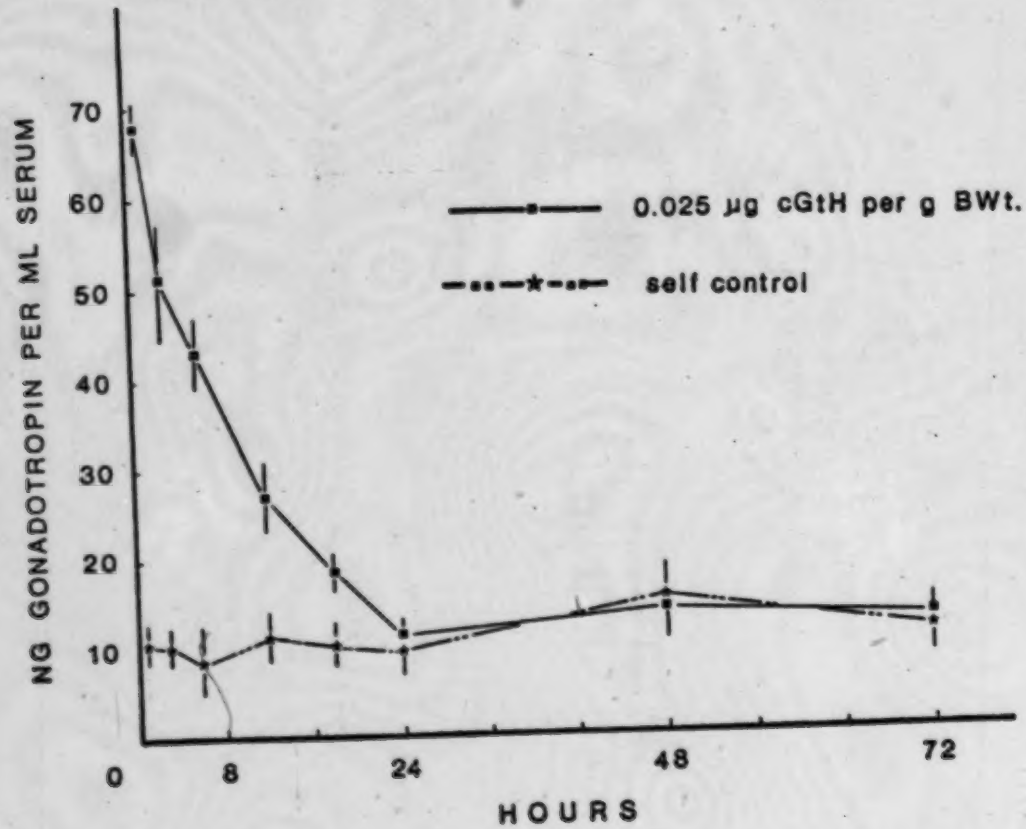


Fig. 4. Serum GtH profiles in sexually mature female goldfish at 12 ± 1° C. The 'self-control' group (solid stars) was sampled 7 days prior to a single intraperitoneal injection of 0.025 µg cGtH per g body weight (solid squares). There was no difference in GSI between groups (pooled GSI = 6.91 ± 0.80%). Values are mean ± SE (N = 8).

Serum Disappearance Profile of Immunoreactive cGtH After a Single Intraperitoneal Injection in Sexually Mature Female Goldfish Maintained at 12°C. GSI = 6.91 ± 0.80 %

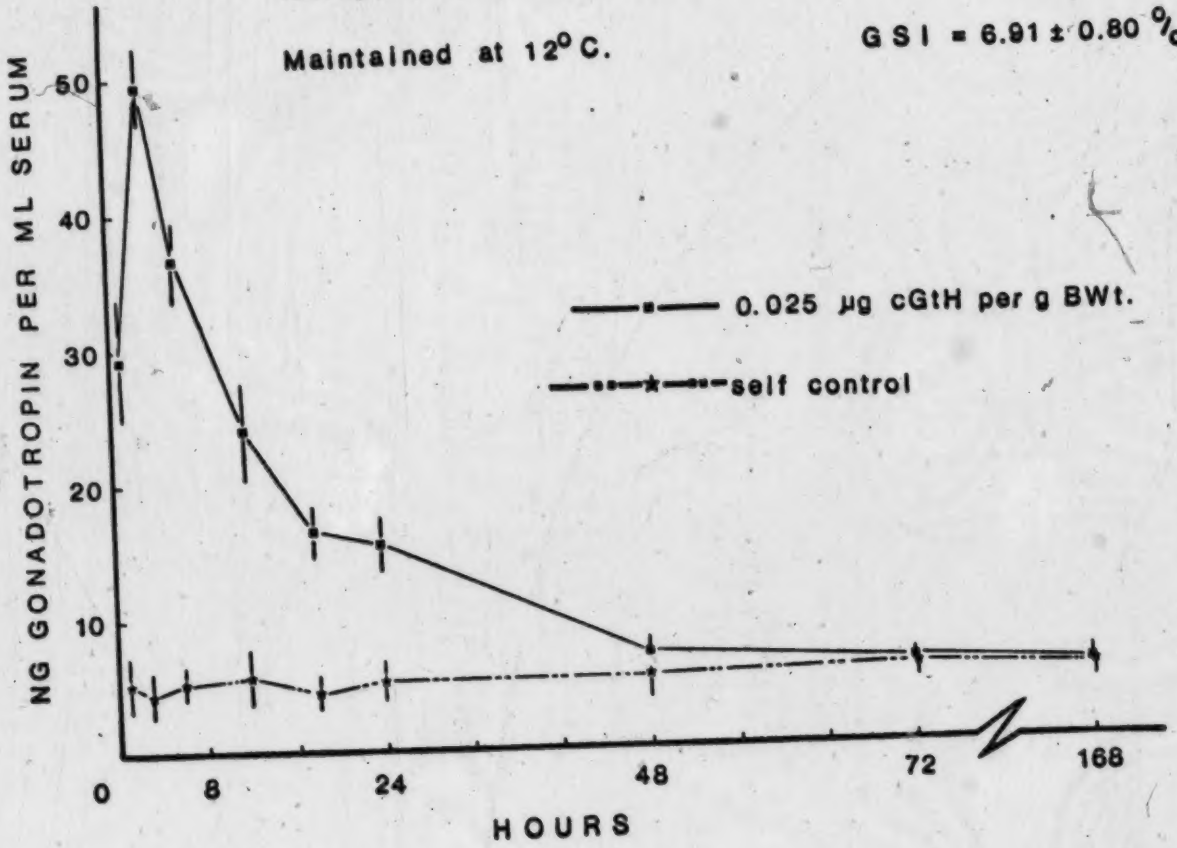
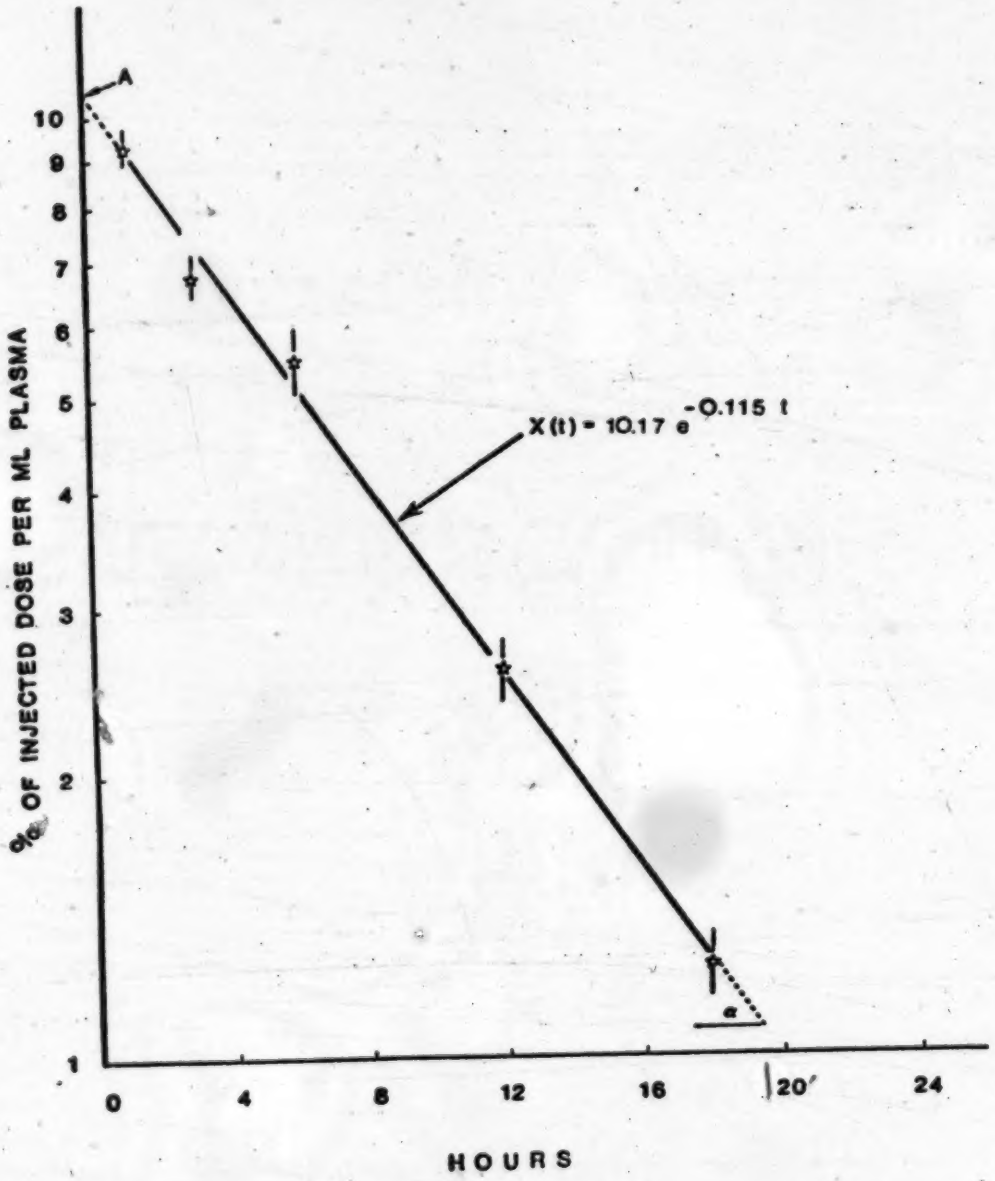


Fig. 5. A semi-logarithmic plot of the metabolic clearance profile of immunoreactive GtH determined in mature male goldfish (GSI = 2.63 ± 0.23%) maintained at 20 ± 1° C. Each value is the mean (vertical bars are ± SE) of 8 to 14 samples obtained by subtracting the initial pre-injection control GtH value from the post-injection sample for each fish, and expressed as a per cent of the injected dose after standardization to a common BWt of 25 g.

Metabolic Clearance Profile of Sexually Mature Male Goldfish

Maintained at T = 20°C. GSI = 2.63 ± 0.23 %



GtH levels declined from the high at 1 hr until at 24 hr post-injection there was no longer a significant difference from the presample values.

The serum GtH profile for sexually mature female goldfish (GSI = 6.91 ± 0.80%) maintained at 12 ± 1° C (Figure 4), is similar to that of mature male goldfish maintained at the same temperature (Figure 2). The maximum serum GtH value (48.42 ± 2.07 ng/ml) was not obtained until 3 hr after the intraperitoneal administration of cGtH. The GtH values of the cGtH injected group remained significantly elevated over the self-control values until 48 hr post-injection. As in the previous two experiments there were no significant differences in the self-control values throughout the sampling period (mean value 5.59 ± 0.38 ng/ml).

The disappearance of exogenous GtH, expressed as a per cent of the administered dose per ml serum, after a single intraperitoneal injection in sexually mature male goldfish maintained at 20 ± 1° C, was characterized by a straight line on a semi-logarithmic plot (Figure 5), indicative of first-order kinetics in a single compartment (Tait and Burstein, 1964). The serum GtH disappearance profiles determined in sexually mature male and female goldfish maintained at 12 ± 1° C also exhibited well-defined single-order kinetics. Table 1 summarizes the results obtained by analysis of the serum disappearance profiles of exogenous GtH, calculated from the data of Figures 2, 3 and 4 after a single intraperitoneal injection in mature male goldfish, maintained at 12 ± 1° C and 20 ± 1° C, and in mature female goldfish at 12 ± 1° C. The observed distribution volume (V_i) obtained from the three experiments suggests a marked effect of

TABLE 1. Comparison of distribution volume, metabolic clearance rate (MCR), half-life ($t_{\frac{1}{2}}$) and pituitary secretion rate (SR) of immunoreactive glycoprotein gonadotropin (GtH) determined using the single injection[a] technique in the goldfish, *Carassius auratus*.

Sex	G.S.I. (%)	Temp. (°C)	Distribution Volume (ml)	Plasma[b] GtH (ng/ml)	Metabolic Clearance Rate (ml/h/25g)	Pituitary[c] Secretion Rate (ng/h/25g)	Half-life[d] (hr)
male	2.63 ± 0.23	20	9.83 ± 1.12	11.63 ± 1.02	1.13 ± 0.18	13.14 ± 2.39	6.03 ± 2.19
male	3.41 ± 0.09	12	14.53 ± 1.15	4.09 ± 0.73	0.98 ± 0.11	4.01 ± 0.84	10.25 ± 0.91
female	6.91 ± 0.80	12	12.50 ± 0.71	5.11 ± 0.61	0.92 ± 0.16	4.70 ± 0.99	9.43 ± 1.39

a A single intraperitoneal injection of 0.025 µg cGtH per g BWt (5 µg cGtH / ml vehicle).

b Mean value determined from all the control plasma GtH values.

c Pituitary SR = Plasma GtH ($ng \cdot ml^{-1}$) x MCR ($ml \cdot h^{-1} \cdot 25g^{-1}$); Tait and Burstein, 1964.

d Half-life = $\frac{0.693}{\alpha}$ (see APPENDIX I).

e All data are mean ± SE; see APPENDIX III.

temperature but no effect of sex. The V_i of males maintained at 12 ± 1° C (14.53 ml) was significantly greater ($p < 0.01$) than at 20 ± 1° C (9.83 ml). The V_i was not significantly different in male and female goldfish maintained at 12 ± 1° C. MCR did not differ significantly with either sex or temperature in these three experiments. However, the $t_{\frac{1}{2}}$ was significantly less ($p < 0.01$) in male goldfish maintained at 20 ± 1° C than at 12 ± 1° C. There was no difference between the $t_{\frac{1}{2}}$ of male and female fish at 12 ± 1° C, the average value being 9.84 ± 0.41 hr. The calculation of pituitary SR indicated a marked effect of temperature on this parameter (Table 1). Since there were no significant differences in the MCR in the three experiments, the elevated plasma levels of endogenous GtH in male goldfish maintained at 20 ± 1° C compared to those at 12 ± 1° C (Table 1) results in a greater than threefold increase in the calculated pituitary SR for the fish at 20 ± 1° C (Table 1).

Part II: Ovarian Uptake of ^{125}I-cGtH and ^{125}I-BSA by Female Goldfish Acclimated to 20 ± 1° C

The uptake of intraarterially injected ^{125}I-cGtH and ^{125}I-BSA with time by the ovaries and other tissues of female goldfish undergoing gonadal recrudescence (GSI = 3.63 ± 0.56%) is shown in Figure 6. There were no significant differences at any sample time in the specific activity of muscle or intestine after the injection of ^{125}I-cGtH compared to ^{125}I-BSA. The ovarian radioactivity increased following the intraarterial injection of ^{125}I-cGtH, reaching a maximum at about 30 minutes, and then declined gradually as shown in

Fig. 6. Comparison of tissue and plasma kinetics of ^{125}I-cGtH (solid diamonds) and ^{125}I-BSA (open diamonds). Sexually maturing female goldfish (GSI = 3.63 ± 0.56%) maintained at 20 ± 1° C received intraarterial injections of either tracer before sacrifice at the times shown. Data are tissue and plasma specific activity expressed as a per cent of the total injected specific activity. A minimum of 5 fish were used for each sample period and all samples were counted in duplicate providing at least ten observations. Values are mean ± SE.

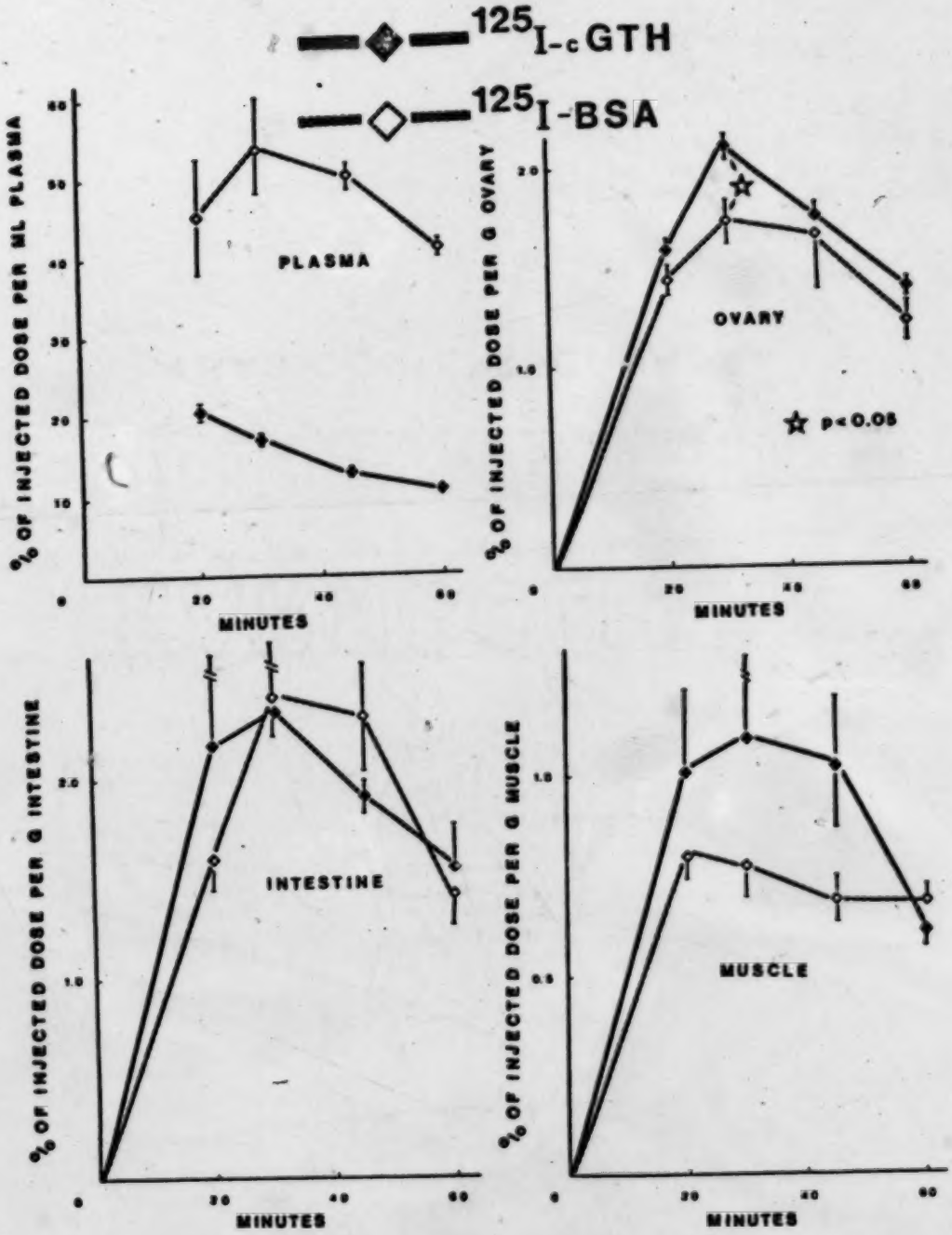
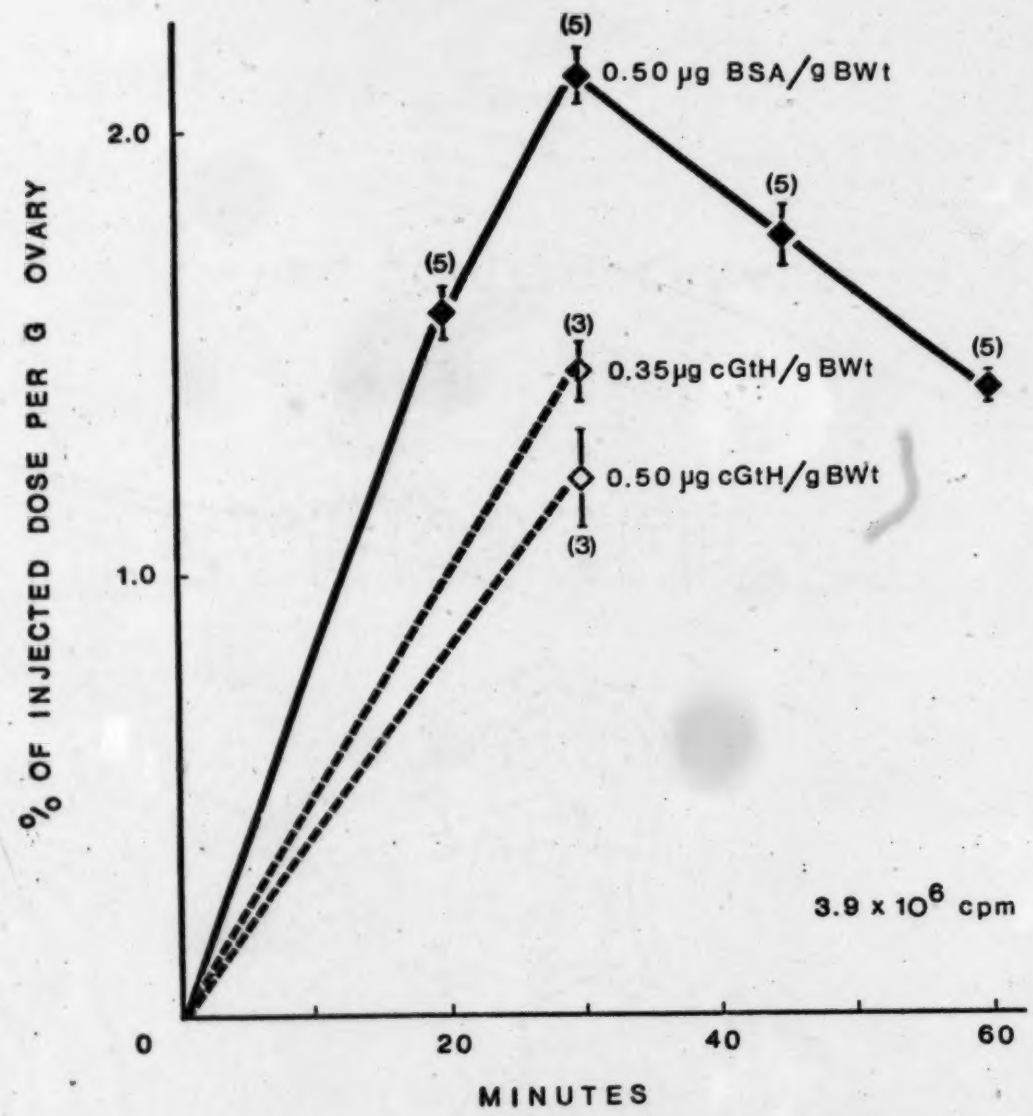

125I-cGTH
125I-BSA
PLASMA
% OF INJECTED DOSE PER ML PLASMA
MINUTES
OVARY
% OF INJECTED DOSE PER G OVARY
☆ p<0.05
INTESTINE
% OF INJECTED DOSE PER G INTESTINE
MUSCLE
% OF INJECTED DOSE PER G MUSCLE

Fig. 7. Sexually maturing female goldfish (GSI = 3.91 ± 0.41%) were acclimated for at least 10 days to a temperature of 20 ± 1° C (12L:12D; lights on 0800 hr) before receiving intraperitoneal injections, at 0800 hr, of either 0.50 µg BSA / g BWt (solid diamonds), 0.35 µg cGtH / g BWt (half-filled diamonds), or 0.50 µg cGtH / BWt (open diamonds). All intraperitoneal injections were made in a volume of 5 µl per g BWt, using saline as a diluent. Three hr later the fish were injected intraarterially with about 1 ng glycoprotein ^{125}I-cGtH (ca. 3.9 x 10^6 cpm). The data shown are mean values of total radioactive counts for individual ovarian samples. The number in parentheses is the number of fish and the vertical bars are ± SE.


(5)
0.50 µg BSA/g BWt
(5)
(5)
(3)
0.35µg cGtH/g BWt
(5)
0.50 µg cGtH/g BWt
(3)
2.0
1.0
0
20
40
60
MINUTES
% OF INJECTED DOSE PER G OVARY
3.9 x 10^6 cpm

Figures 6 and 7. The specific activity of ovarian tissue was significantly greater at 30 minutes in the ^{125}I-cGtH injected fish than in fish injected with ^{125}I-BSA. In addition, the time course of disappearance of plasma specific activity after the injection of ^{125}I-BSA was very different than that observed after the intra-arterial injection of ^{125}I-cGtH. Plasma radioactivity of ^{125}I-BSA was more than double that of ^{125}I-cGtH at all sample times (Figure 6), and the plasma disappearance of the foreign protein tracer did not resemble any obvious negative exponential, in contrast to the plasma disappearance of ^{125}I-cGtH (Figure 6 and see Part III of RESULTS). This system for determining the ovarian uptake of labelled cGtH provides the basis for experiments to determine whether unlabelled cGtH can significantly compete with ^{125}I-cGtH for ovarian uptake.

When maturing female goldfish (GSI = 3.91 ± 0.41%) were pre-injected with either of two doses of unlabelled cGtH, ovarian uptake of radioactivity was significantly decreased ($p < 0.01$) compared to those fish which were pre-injected with an equivalent amount of BSA (Figure 7). Furthermore, the amount of inhibition was positively related to the dose of unlabelled cGtH (Figure 7). Pre-injection of 0.50 µg BSA per g BWt did not result in decreased ovarian binding of ^{125}I-cGtH (Figure 7) compared to those fish which did not receive a pre-injection (Figure 6). The finding that small quantities of unlabelled hormone effectively compete with the binding of ^{125}I-cGtH indicates that much of the ^{125}I-cGtH was bound at physiologically significant sites, rather than adsorbed nonspecifically to the ovary.

Part III: Clearance of Intraarterially Injected ^{125}I-cGtH

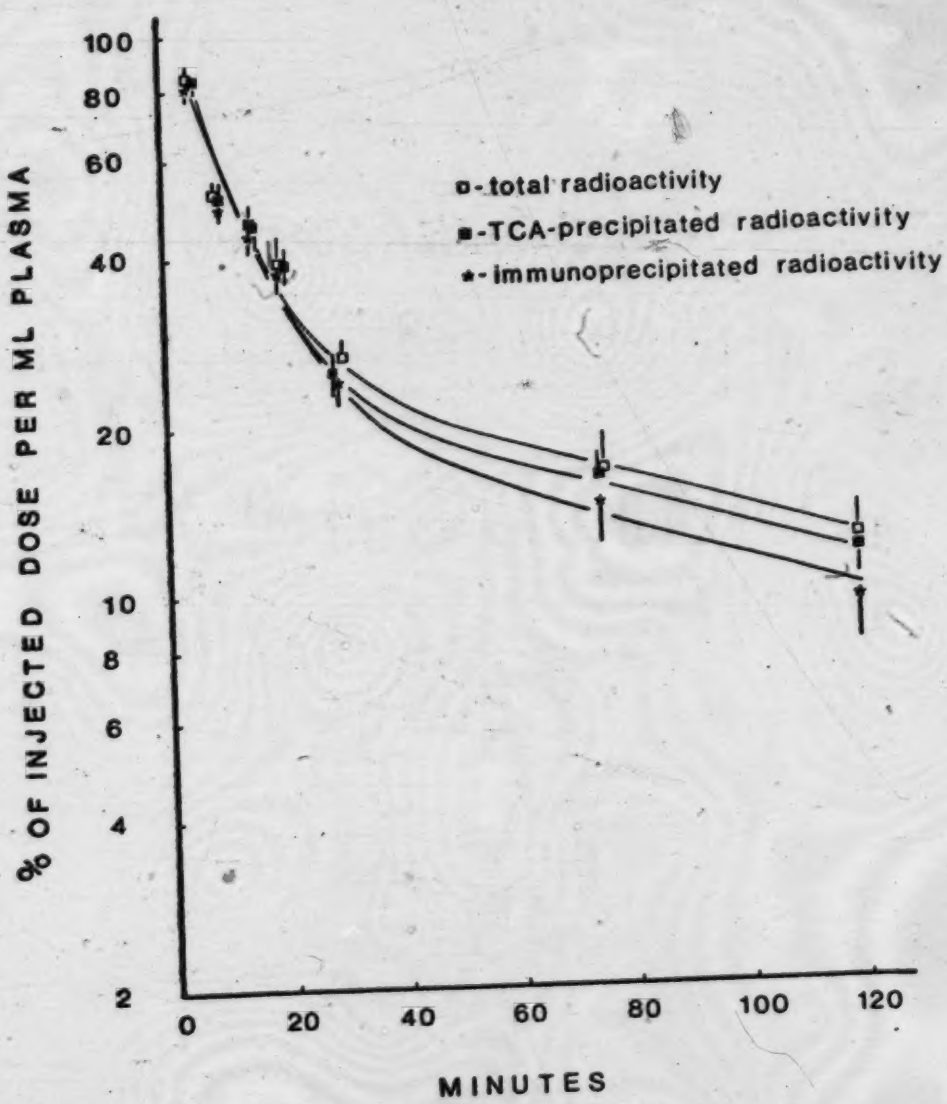
In the clearance experiments, 77.21 ± 2.64 (mean ± SE) per cent of the ^{125}I-cGtH in the injection volume was immunoprecipitable with 'excess' RAC antisera. This value is similar to that reported for a number of radioiodinated mammalian gonadotropins (Akbar *et al.*, 1974; Koch *et al.*, 1971; Rizkallah *et al.*, 1969). Also, the immunoprecipitable counts in the injection solution did not vary over the 5 days during which each clearance experiment was performed (data not shown). Figure 1 demonstrates the suitability of the antisera dilution used for measuring ^{125}I-cGtH in the plasma of intact goldfish. Concentrations of up to 75 ng GtH per ml plasma did not interfere with the immunoprecipitation of ^{125}I-cGtH over the range of plasma specific activities encountered throughout the 120 minute sampling period of the clearance studies.

The plasma disappearance curves of intraarterially injected glycoprotein ^{125}I-cGtH plotted on semi-logarithmic coordinates show the characteristics of multiexponential curves in all experiments (Figures 8, 9, 10, 12, 13 and 14). The kinetics of disappearance of ^{125}I-cGtH from the plasma of goldfish in these experiments is accurately described by equation 2 (p. 21, METHODS AND MATERIALS). Eighty-nine ± 1.87 (mean ± SE for all intraarterial ^{125}I-cGtH clearance experiments) per cent of the variance of the plasma radioactive values is accounted for by the fitted double exponential curves. The mean value for the standard error of the estimated values from all experiments is 6.01 ± 1.00 (mean ± SE) per cent of

Fig. 8. Metabolic clearance profiles of ^{125}I-cGtH in sexually regressed female goldfish (GSI = 1.70 ± 0.35%) maintained at 12 ± 1° C. Values are mean ± SE (N = 5).

Metabolic Clearance Profiles of Regressed Females at T = 12°C.

GSI = 1.70 ± 0.35%



injected dose per ml plasma. A two compartment model contains the minimum number of pools consistent with the experimental data. However, it is difficult to establish a physiologically significant counterpart for the second pool; further this type of experimental data may be equally compatible with models composed of multiple pools which may better describe the physiologic situation. Therefore, although the various kinetic parameters applicable to the two-pool system may be calculated (Gurpide, 1975; Shipley and Clark, 1972; Tait and Burstein, 1964) from the ^{125}I-cGtH plasma clearance data, these values are not reported herein. The calculated values of overall MCR and pituitary SR may be obtained without making assumptions about the number and nature of the pools exchanging with the circulating ^{125}I-cGtH (see METHODS AND MATERIALS and APPENDIX II). The values of $t_{\frac{1}{2}_i}$ and primary MCR which do necessitate a two-pool system are included to facilitate comparison with mammalian hormone clearance studies.

Fish with regressed gonads

The plasma MCP's of total, TCA-precipitated and immunoprecipitated radioactive measurements of glycoprotein ^{125}I-cGtH determined in sexually regressed female goldfish (GSI = 1.70 ± 0.35%) maintained at 12 ± 1° C are very similar (Figure 8). The lack of significant differences between the three methods of assay suggest that only a small amount of radioiodinated degradation products are produced over the 2 hr sampling period. As expected, there are few differences between the methods of assay in the calculated

TABLE 2. Comparison of distribution volume of the first compartment, metabolic clearance rate (MCR) and initial half-life of glycoprotein ^{125}I-cGtH calculated from measurements of total, TCA-precipitated (TCA) and immunoprecipitated (Immuno) plasma radioactivity in sexually regressed female goldfish (GSI = 1.70 ± 0.35%), *Carassius auratus*, at T = 12 ± 1° C shown in Figure 8.

Plasma radioactivity assayed	Distribution volume [a] ml	MCR (ml · h^{-1} · 25g^{-1}) Primary [b]	MCR (ml · h^{-1} · 25g^{-1}) Overall [c]	Initial half-life [d] min
Total	0.59 ± 0.04 [e]	10.44 ± 0.32	1.45 ± 0.05	13.07 ± 1.53
TCA	0.60 ± 0.34	10.41 ± 0.34	1.49 ± 0.06	12.07 ± 2.20
Immuno	0.56 ± 0.04	10.88 ± 0.32	1.66 ± 0.05	12.83 ± 1.74

a Distribution volume of first compartment computed as $(A + B)^{-1}$ (Tait and Burstein, 1964).

b Primary MCR computed as $\alpha \cdot A^{-1}$ (Campbell *et al.*, 1978a; APPENDIX II).

c Overall MCR evaluated by the methods of Tait and Burstein (1964); see APPENDIX II.

d Initial biological half-life, evaluated according to Shipley and Clark (1972); see APPENDIX I.

e All data are mean ± SE; see APPENDIX III.

clearance or distribution parameters (Table 2). The average value for V_i in this experiment is 0.58 ± 0.01 ml and represents approximately 2.3 per cent of the standard 25 g BWt. The overall MCR was significantly greater ($p < 0.01$) when calculated from the immunoprecipitated MCP compared to that calculated from either the total or TCA-precipitated radioactivity MCP's. The average value of primary MCR (10.58 ± 0.15 $ml \cdot h^{-1} \cdot 25g$) is much greater than the overall MCR (Table 2) since the calculation of the former is evaluated from the area beneath the initial portion of the MCP defined by the first rate constant α and ordinant intercept A. The calculated values of $t_{\frac{1}{2}_i}$ also did not differ significantly with method of assay in regressed females maintained at 12 ± 1° C (Table 2), the average value being 12.66 ± 0.30 minutes.

The multiexponential MCP's determined in sexually regressed female goldfish (GSI = 1.15 ± 0.07%) acclimated to 20 ± 1° C (Figure 9) are quite different than for fish acclimated to 12 ± 1° C (Figure 8). At 20 ± 1° C it is apparent that the disappearance curve of total radioactivity tends to deviate from that of TCA-precipitated radioactivity which in turn deviates from that of the immunoprecipitated radioactivity (Figure 9), presumably reflecting the appearance in the plasma of significant amounts of radioiodinated degradation products. The immunoprecipitable fraction of the total radioactivity in plasma obtained 120 minutes post-injection, decreased to less than 50 per cent of the value present in the injected material. As a check of nonspecific effects of plasma upon degradation of ^{125}I-cGtH, an aliquot of the injection solution was

Fig. 9. Metabolic clearance profiles of ^{125}I-cGtH in sexually regressed female goldfish (GSI = 1.15 ± 0.07%) maintained at 20 ± 1° C. Values are mean ± SE (N = 5).

Metabolic Clearance Profiles of Regressed Females at T = 20° C

GSI = 1.15 ± 0.07 %

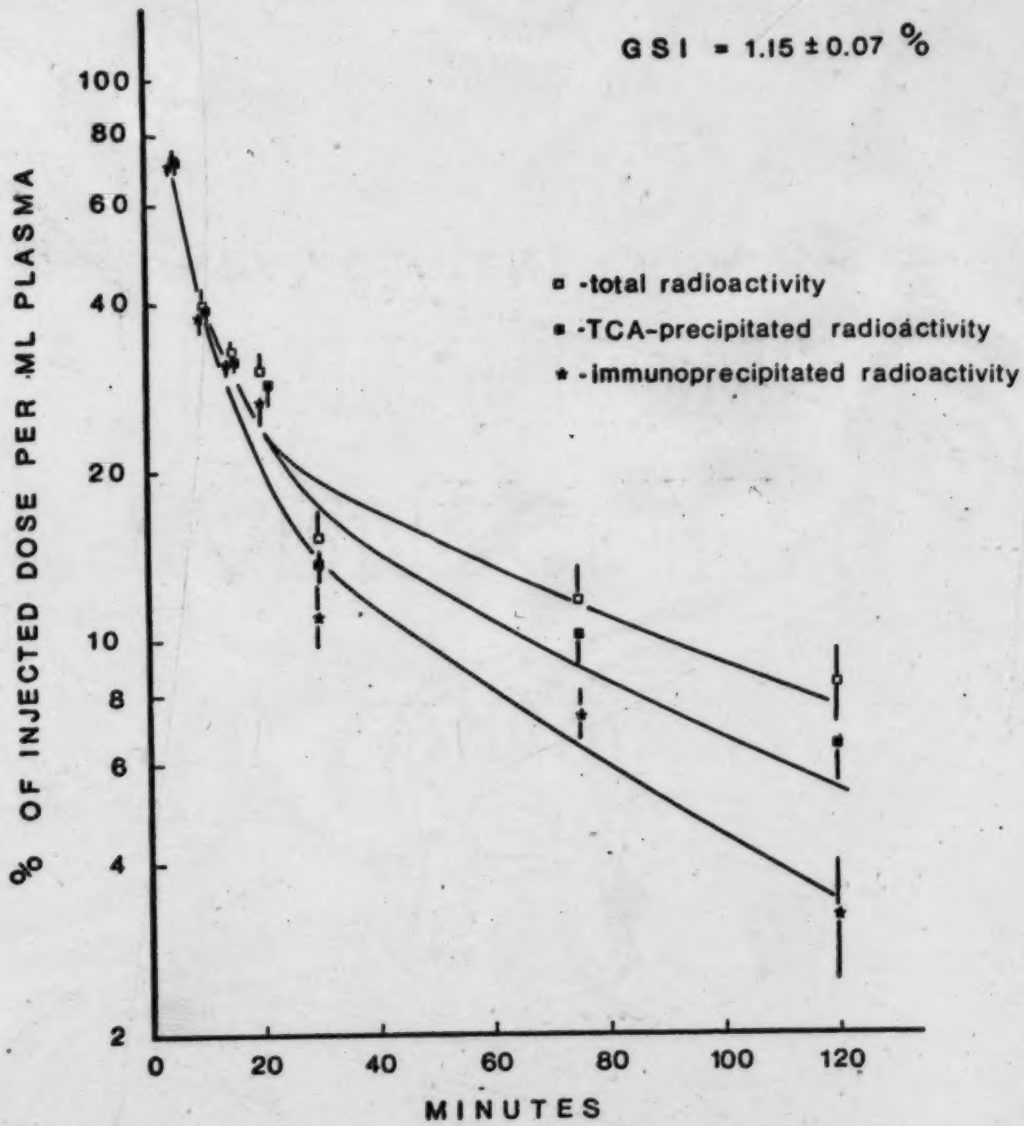


TABLE 3. Comparison of distribution volume of the first compartment, metabolic clearance rate (MCR) and initial half-life of glycoprotein ^{125}I-cGtH calculated from measurements of total, TCA-precipitated (TCA) and immunoprecipitated (Immuno) plasma radioactivity in sexually regressed female goldfish (GSI = 1.15 ± 0.07%), *Carassius auratus*, at T = 20 ± 1° C shown in Figure 9.

Plasma radioactivity assayed	Distribution volume[a] ml	MCR (ml · h^{-1} · 25g^{-1}) Primary[b]	MCR (ml · h^{-1} · 25g^{-1}) Overall[c]	Initial half-life[d] min
Total	0.59 ± 0.02[e]	8.60 ± 0.12	1.86 ± 0.05	10.09 ± 0.55
TCA	0.57 ± 0.02	8.91 ± 0.13	2.16 ± 0.01	10.36 ± 0.59
Immuno	0.56 ± 0.09	9.71 ± 0.48	2.76 ± 0.53	11.67 ± 0.62

a Distribution volume of first compartment computed as $(A + B)^{-1}$ (Tait and Burstein, 1964).

b Primary MCR computed as $\alpha \cdot A^{-1}$ (Campbell *et al.*, 1978a; APPENDIX II).

c Overall MCR evaluated by the methods of Tait and Burstein (1964); see APPENDIX II.

d Initial biological half-life, evaluated according to Shipley and Clark (1972); see APPENDIX I.

e All data are mean ± SE; see APPENDIX III.

Fig. 10. Metabolic clearance profiles of ^{125}I-cGtH in sexually regressed male goldfish (GSI = 0.75 ± 0.10%) maintained at 20 ± 1° C. Values are mean ± SE (N = 5).

Metabolic Clearance Profiles of Regressed Males at T = 20°C

GSI = 0.75 ± 0.10 %

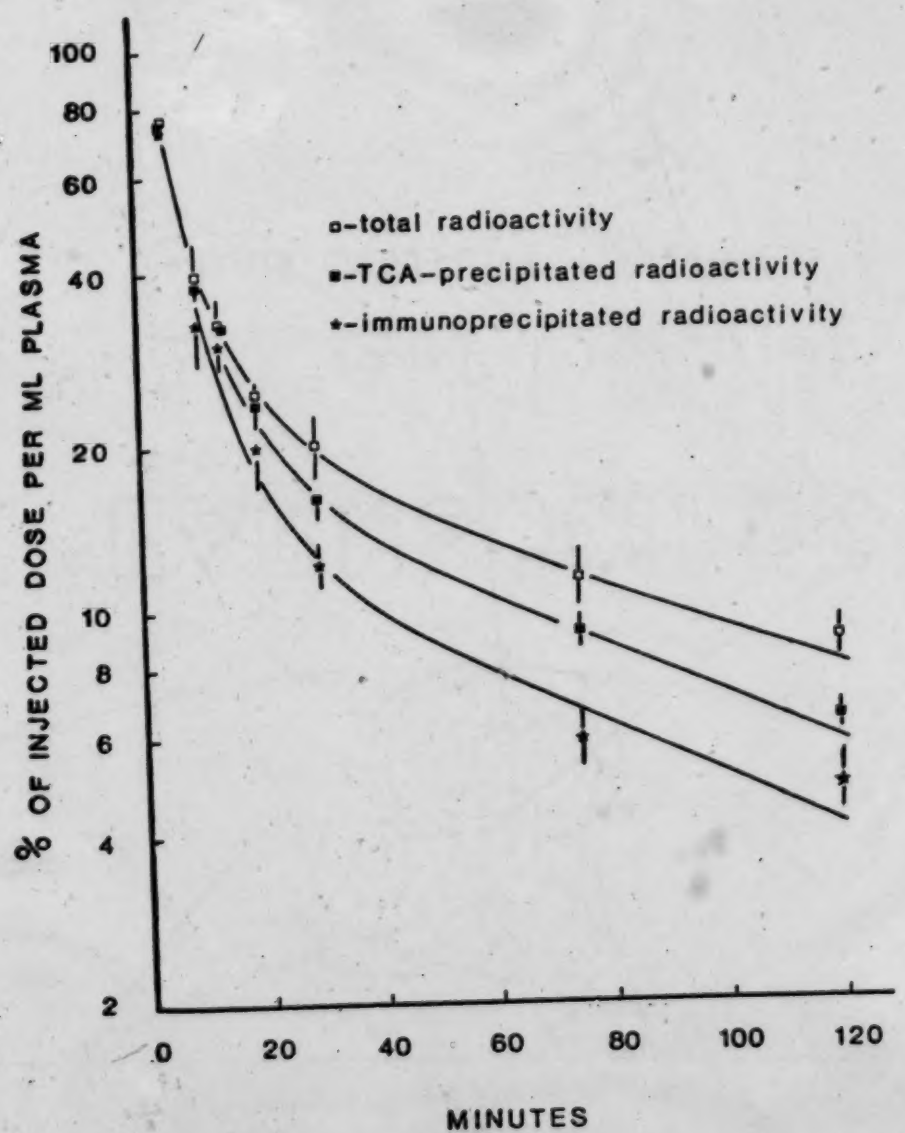


TABLE 4. Comparison of distribution volume of the first compartment, metabolic clearance rate (MCR) and initial half-life of glycoprotein ^{125}I-cGtH calculated from measurements of total, TCA-precipitated (TCA) and immunoprecipitated (Immuno) plasma radioactivity in sexually regressed male goldfish (GSI = 0.75 ± 0.10%), *Carassius auratus*, at T = 20 ± 1° C shown in Figure 10.

Plasma radioactivity assayed	Distribution volume [a] ml	MCR (ml · h^{-1} · 25g^{-1}) Primary [b]	MCR (ml · h^{-1} · 25g^{-1}) Overall [c]	Initial half-life [d] min
Total	0.60 ± 0.03 [e]	9.08 ± 0.09	1.90 ± 0.03	7.40 ± 0.51
TCA	0.57 ± 0.02	9.60 ± 0.13	2.26 ± 0.12	5.78 ± 0.74
Immuno	0.66 ± 0.02	8.27 ± 0.15	2.63 ± 0.01	7.03 ± 0.56

a Distribution volume of first compartment computed as $(A + B)^{-1}$ (Tait and Burstein, 1964).

b Primary MCR computed as $\alpha \cdot A^{-1}$ (Campbell *et al.*, 1978a; APPENDIX II).

c Overall MCR evaluated by the methods of Tait and Burstein (1964); see APPENDIX II.

d Initial biological half-life, evaluated according to Shipley and Clark (1972); see APPENDIX I.

e All data are mean ± SE; see APPENDIX III.

incubated in plasma at 20° C *in vitro* for 120 minutes. The loss of immunologic activity amounted to 3 per cent. It is then not surprising to note considerable differences in the clearance parameters with method of assay in the regressed female goldfish at 20 ± 1° C (Table 3). The overall MCR, when determined by the immunoprecipitation method is 150 per cent greater than the corresponding value calculated from the total radioactivity MCP (Table 3). In addition, the primary MCR calculated from the immunoprecipitated MCP is significantly greater ($p < 0.05$) than that evaluated from total radioactivity. The initial $t_{\frac{1}{2}_i}$ was significantly greater ($p < 0.01$) when computed using the immunoprecipitated MCP (11.67 ± 0.62 minutes) compared to either the total or TCA-precipitated MCP calculated $t_{\frac{1}{2}_i}$'s (Table 3). From this it is clear that the exclusion of non-immunoprecipitable radioiodinated degradation products is important for the accurate determination of ^{125}I-cGtH plasma clearance parameters in sexually regressed female goldfish acclimated to 20 ± 1° C. The calculated values of V_i do not vary significantly with method of assay, the average value being 0.57 ± 0.01 ml or about 2.3 per cent of the total BWt.

Figure 10 shows the MCP's determined from sexually regressed male goldfish (GSI = 0.75 ± 0.10%) maintained at 20 ± 1° C. Figure 11 illustrates the 'curve-peeling' method of graphical analysis (Shipley and Clark, 1972; see METHODS AND MATERIALS) with the immunoprecipitated plasma metabolic clearance profile from regressed males at 20 ± 1° C (same experiment as Figure 10 and Table 4). Table 4 summarizes the results obtained from this experiment. Similar

Fig. 11. Metabolic clearance profile (MCP) of immunoprecipitated ^{125}I-cGtH determined in sexually regressed male goldfish at 20 ± 1° C illustrating the 'curve-peeling' method of graphical analysis (Shipley and Clark, 1972; and see METHODS AND MATERIALS). The solid stars are mean ± 2SE (N = 5). The open stars are points obtained by the subtraction of the values at 5, 10, 15, 20 and 30 minutes on the line of slope β and intercept B when extrapolated to t = 0, from the initial part of the MCP between 5 and 30 minutes. The shaded area represents the 95 per cent confidence interval of the curve described by x(t).

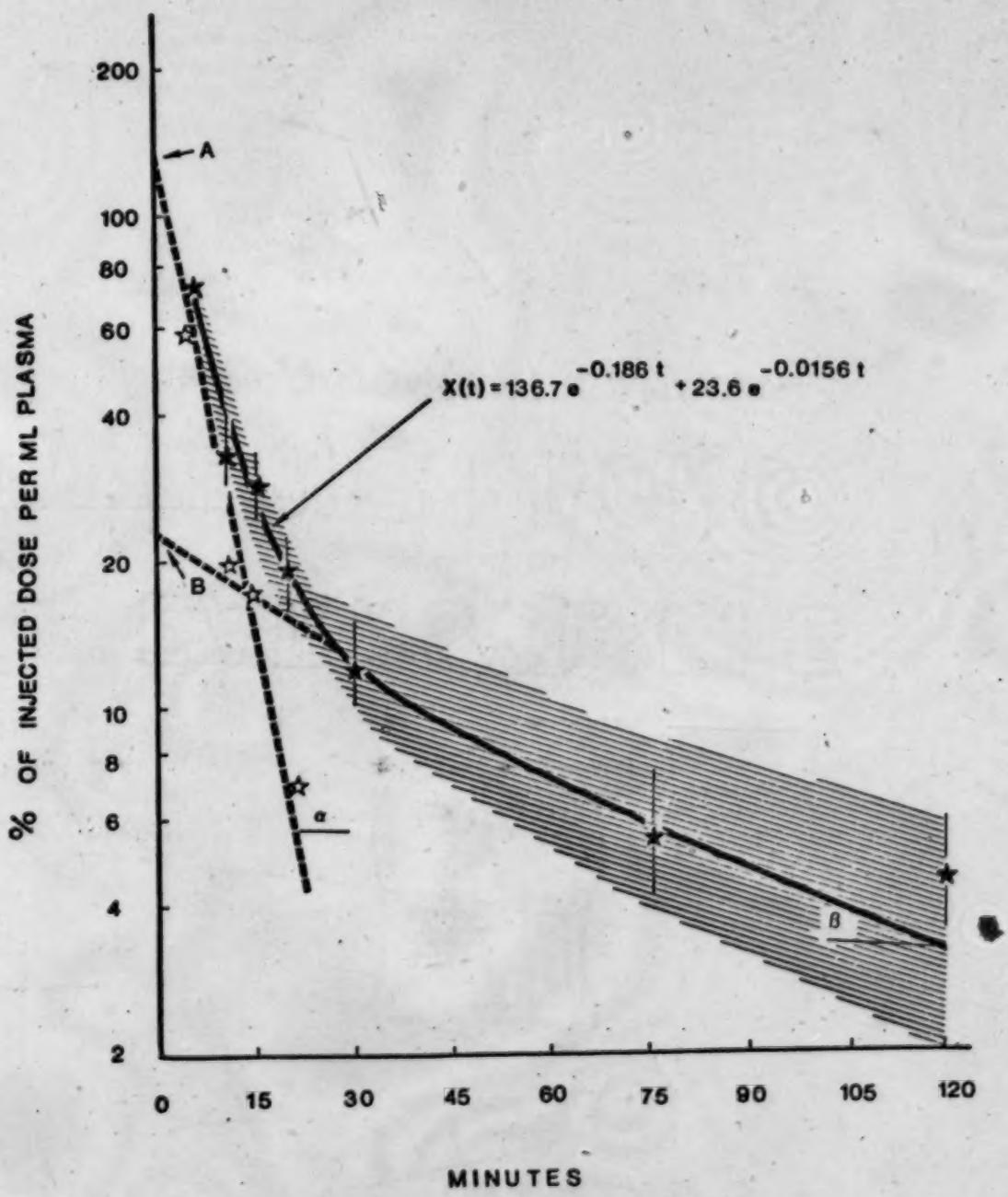

200
100
80
60
40
20
10
8
6
4
2
A
B
α
β
X(t) = 136.7 e^-0.186 t + 23.6 e^-0.0156 t
% OF INJECTED DOSE PER ML PLASMA
0
15
30
45
60
75
90
105
120
MINUTES

Fig. 12. Metabolic clearance profiles of ^{125}I-cGtH in sexually maturing female goldfish (GSI = 4.74 ± 0.45%) maintained at 12 ± 1° C. Values are mean ± SE (N = 5).

Metabolic Clearance Profiles of Maturing Females at T = 12°C

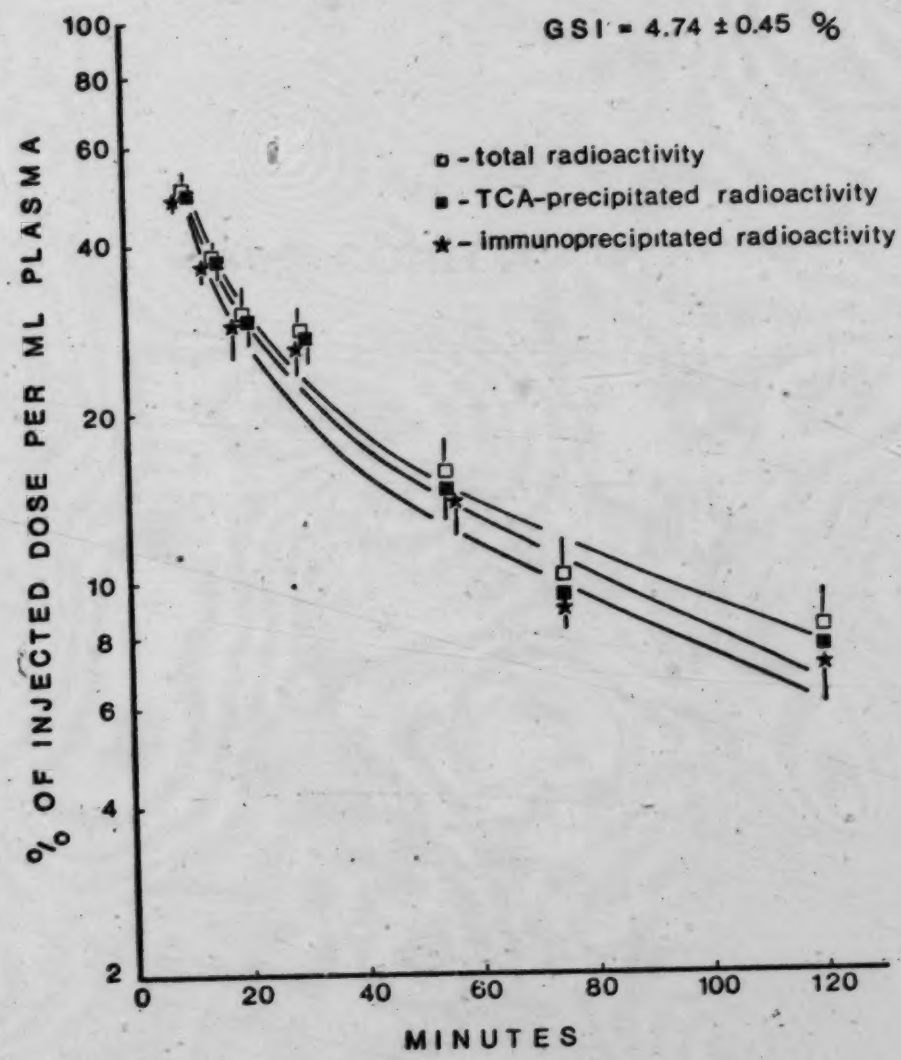


TABLE 5. Comparison of distribution volume of the first compartment, metabolic clearance rate (MCR) and initial half-life of glycoprotein ^{125}I-cGtH calculated from measurements of total, TCA-precipitated (TCA) and immunoprecipitated (Immuno) plasma radioactivity in sexually maturing female goldfish (GSI = 4.74 ± 0.45%), *Carassius auratus*, at T = 12 ± 1° C shown in Figure 12.

Plasma radioactivity assayed	Distribution volume [a] ml	MCR (ml · h^{-1} · 25g^{-1}) Primary [b]	Overall [c]	Initial half-life [d] min
Total	0.49 ± 0.09 [e]	8.83 ± 1.03	1.89 ± 0.05	2.28 ± 0.31
TCA	0.39 ± 0.09	7.82 ± 1.28	1.97 ± 0.09	2.56 ± 0.31
Immuno	0.64 ± 0.07	9.83 ± 0.50	1.93 ± 0.03	3.65 ± 0.30

a Distribution volume of first compartment computed as $(A + B)^{-1}$ (Tait and Burstein, 1964).

b Primary MCR computed as $\alpha \cdot A^{-1}$ (Campbell *et al.*, 1978a; APPENDIX II).

c Overall MCR evaluated by the methods of Tait and Burstein (1964); see APPENDIX II.

d Initial biological half-life, evaluated according to Shipley and Clark (1972); see APPENDIX I.

e All data are mean ± SE; see APPENDIX III.

to the regressed females maintained at the same temperature, the overall MCR in regressed males at 20 ± 1° C computed from the immunoprecipitated plasma radioactivity is significantly greater ($p < 0.01$) than that calculated from either total or TCA-precipitated plasma radioactivity. The primary MCR determined from the immunoprecipitated MCP in regressed male goldfish is less than that calculated for regressed female goldfish at 20 ± 1° C, but there are no differences in the overall MCR between the two groups. The reason for the difference in initial MCR is perhaps related to the significantly greater calculated value of V_i and the faster $t_{\frac{1}{2}i}$ in the males, based on immunoprecipitation of plasma ^{125}I-cGtH (Table 4).

Fish undergoing ovarian recrudescence

The plasma MCP's of total, TCA-precipitated and immunoprecipitated radioactive measurements of glycoprotein ^{125}I-cGtH in female goldfish undergoing ovarian recrudescence are of the usual multiexponential nature at 12 ± 1° C (Figure 12) and 20 ± 1° C (Figure 13). The comparison of V_i, MCR and $t_{\frac{1}{2}i}$ determined from the three methods of assay for maturing females at 12 ± 1° C (GSI = 4.74 ± 0.45%; Table 5) and at 20 ± 1° C (GSI = 5.02 ± 0.35%; Table 6) suggest that only a small amount of degradation of the injected ^{125}I-cGtH occurred under each condition. The overall MCR did not vary significantly with method of assay at either 12 ± 1° C or 20 ± 1° C (Tables 5 and 6). Further, there was no detectable effect of temperature on overall MCR. In contrast, the values of primary MCR, determined from all assay methods, were significantly

Fig. 13. Metabolic clearance profiles of ^{125}I-cGtH in sexually maturing female goldfish (GSI = 5.02 ± 0.35%) maintained at 20 ± 1° C. Values are mean ± SE (N = 5).

Metabolic Clearance Profiles of Maturing Females at T = 20°C

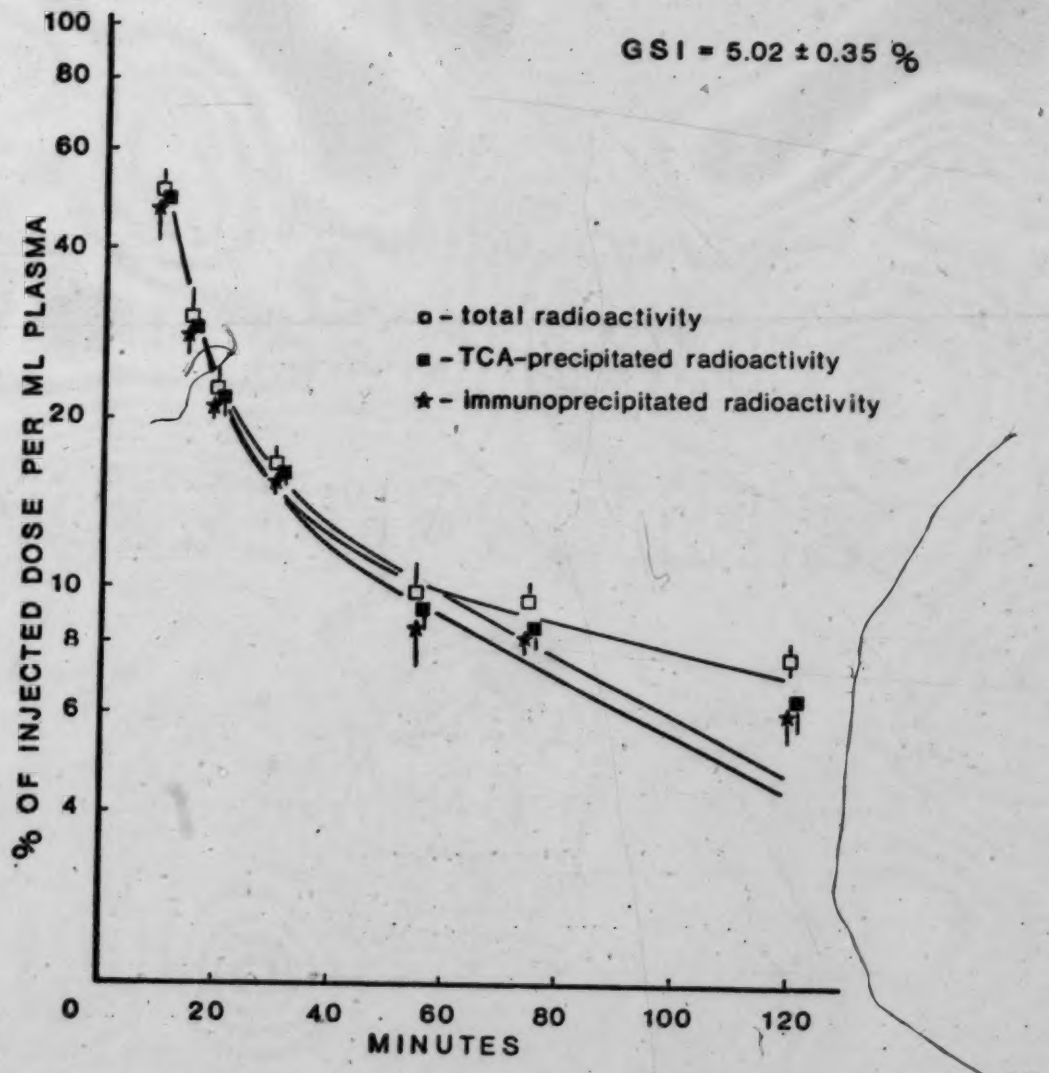


TABLE 6. Comparison of distribution volume of the first compartment, metabolic clearance rate (MCR) and initial half-life of glycoprotein ^{125}I-cGtH calculated from measurements of total, TCA-precipitated (TCA) and immunoprecipitated (Immuno) plasma radioactivity in sexually maturing female goldfish (GSI = 5.02 ± 0.35%), *Carassius auratus*, at T = 20 ± 1° C shown in Figure 13.

Plasma radioactivity assayed	Distribution volume [a] ml	MCR (ml · h^{-1} · 25g^{-1}) Primary [b]	MCR (ml · h^{-1} · 25g^{-1}) Overall [c]	Initial half-life [d] min
Total	0.55 ± 0.05 [e]	5.85 ± 0.19	1.84 ± 0.09	4.33 ± 0.27
TCA	0.41 ± 0.05	5.20 ± 0.29	2.11 ± 0.08	3.65 ± 0.30
Immuno	0.42 ± 0.05	5.36 ± 0.30	2.09 ± 0.08	3.57 ± 0.28

a Distribution volume of first compartment computed as $(A + B)^{-1}$ (Tait and Burstein, 1964).

b Primary MCR computed as $\alpha \cdot A^{-1}$ (Campbell *et al.*, 1978a; APPENDIX II).

c Overall MCR evaluated by the methods of Tait and Burstein (1964); see APPENDIX II.

d Initial biological half-life, evaluated according to Shipley and Clark (1972); see APPENDIX I.

e All data are mean ± SE; see APPENDIX III.

greater for the maturing female goldfish at 12 ± 1° C than for the 20 ± 1° C acclimated fish. The values of V_1 were not significantly different with method of assay for the maturing female goldfish maintained at 20 ± 1° C (Table 6). The mean value for V_1 calculated from all assay methods for the maturing female goldfish at both 12 ± 1° C and 20 ± 1° C represents about 2 per cent of the total BWt of the standard 25 g fish. The initial $t_{\frac{1}{2}i}$ was significantly greater ($p < 0.01$) in female goldfish maintained at 20 ± 1° C than at 12 ± 1° C, when calculated from either the total or TCA-precipitated radioactivity MCP's. However, there was no statistical effect of temperature on the values of $t_{\frac{1}{2}i}$ when calculated from the immunoprecipitated radioactivity MCP's.

Fish with a mature ovary

The plasma MCP's of total, TCA-precipitated and immunoprecipitated radioactive measurements of glycoprotein ^{125}I-cGtH in sexually mature female goldfish (GSI = 15.41 ± 0.64%) are multiexponential in nature (Figure 14). Similar to results obtained from sexually regressed fish maintained at 20 ± 1° C, but in contrast to sexually maturing female goldfish, results obtained on the mature females at 12 ± 1° C using the three methods of assay suggest the appearance in the plasma of significant amounts of radioiodinated degradation products. The value of overall MCR computed from the immunoprecipitated MCP is significantly greater ($p < 0.01$) than that calculated from either total or TCA-precipitated radioactivity assay methods (Table 7). Similarly the values of primary MCR, V_1

Fig. 14. Metabolic clearance profiles of ^{125}I-cGtH in sexually mature female goldfish (GSI = 15.41 ± 0.64%) maintained at 12 ± 1° C. Values are mean ± SE (N = 5).

Metabolic Clearance Profiles of Mature Females T=12°C

GSI =15.41±0.64 %

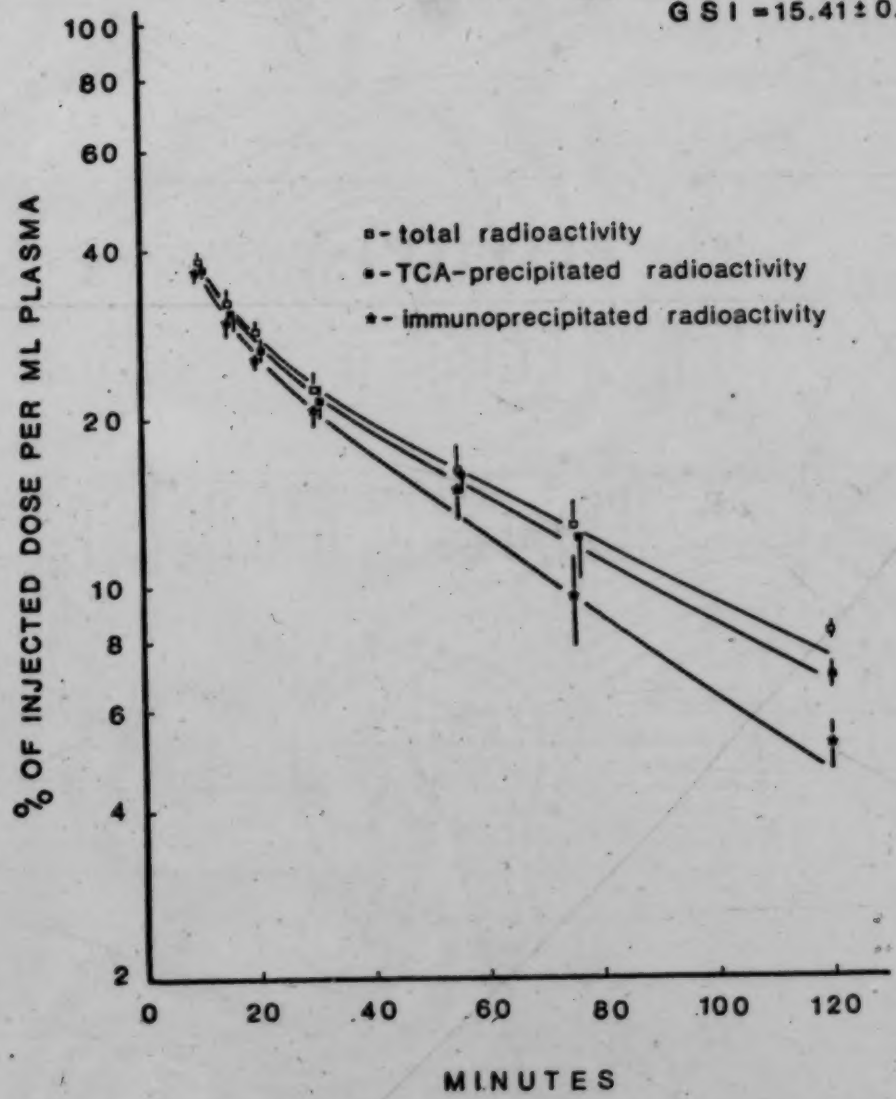


TABLE 7. Comparison of distribution volume of the first compartment, metabolic clearance rate (MCR) and initial half-life of glycoprotein ^{125}I-cGtH calculated from measurements of total, TCA-precipitated (TCA) and immunoprecipitated (Immuno) plasma radioactivity in sexually mature female goldfish (GSI = 15.41 ± 0.64%), *Carassius auratus*, at T = 12 ± 1° C shown in Figure 14.

Plasma radioactivity assayed	Distribution volume [a] ml	MCR (ml · h^{-1} · 25g^{-1}) Primary [b]	Overall [c]	Initial half-life [d] min
Total	1.70 ± 0.05 [e]	17.18 ± 1.50	2.03 ± 0.04	8.04 ± 0.77
TCA	1.02 ± 0.21	20.87 ± 3.30	2.39 ± 0.04	3.11 ± 0.50
Immuno	1.36 ± 0.14	23.76 ± 1.47	2.52 ± 0.03	4.38 ± 0.66

a Distribution volume of first compartment computed as $(A + B)^{-1}$ (Tait and Burstein, 1964).

b Primary MCR computed as $\alpha \cdot A^{-1}$ (Campbell *et al.*, 1978a; APPENDIX II).

c Overall MCR evaluated by the methods of Tait and Burstein (1964); see APPENDIX II.

d Initial biological half-life, evaluated according to Shipley and Clark (1972); see APPENDIX I.

e All data are mean ± SE; see APPENDIX III.

and $t_{\frac{1}{2}_i}$ are markedly affected by the method of assay. These data reemphasize the necessity of the immunoprecipitation method to minimize the contribution of radioiodinated degradation products of GtH in the calculation of clearance and distribution parameters. The data shown in Table 7 illustrates two striking differences in the clearance and distribution of ^{125}I-cGtH in sexually mature female goldfish acclimated to 12 ± 1° C compared to both regressed and maturing female goldfish. The value of primary MCR determined from sexually mature fish, by the immunoprecipitated method of assay, is more than 9 times greater the overall MCR value (Table 7). The previous experiments indicated that values of primary MCR determined by the immunoprecipitated assay method varied between 2.6 and 6.6 times the value of overall MCR. Since the values of MCR and $t_{\frac{1}{2}_i}$ determined from the immunoprecipitated MCP's of mature female fish (Table 7) are similar to values determined in sexually maturing goldfish (Tables 5 and 6), it is likely the greater estimates of V_i in sexually mature goldfish acclimated to 12 ± 1° C are a contributing factor. The value of V_i (1.36 ± 0.14 ml) calculated from the immunoprecipitated assay method represents about 5.4 per cent of the BWt of a standard 25 g mature female goldfish maintained at 12 ± 1° C.

Table 8 provides a summary comparison of results obtained from analysis of the MCP's of immunoprecipitated ^{125}I-cGtH, plasma GtH levels and calculated values of the pituitary SR of GtH from all intraarterial injection experiments described above. The GSI of the sexually mature fish group was found to be significantly greater ($p < 0.01$) than the GSI of both the 12 ± 1° C and 20 ± 1° C

TABLE 8. Comparison of some gonadotropin parameters determined from analysis of metabolic clearance profiles of immunoprecipitated ^{125}I-cGtH, in the goldfish, *Carassius auratus*.

		Males	Females	Females	Females	Females	Females
GSI	(%)	0.75±0.10	1.15±0.07	1.70±0.35	4.74±0.45	5.02±0.35	15.41±0.64
Temp	(°C)	20±1	20±1	12±1	12±1	20±1	12±1
V_1	(ml)	0.66±0.02[a]	0.56±0.09	0.56±0.04	0.64±0.07	0.42±0.05	1.36±0.14
$t_{\frac{1}{2}_1}$	(min)	7.03±0.56	11.67±0.62	12.83±1.74	3.65±0.30	3.57±0.28	4.38±0.66
MCR primary	($ml \cdot h^{-1} \cdot 25g^{-1}$)	8.23±0.15	9.71±0.48	10.88±0.32	9.83±0.50	5.36±0.30	23.76±1.47
MCR overall	($ml \cdot h^{-1} \cdot 25g^{-1}$)	2.63±0.01	2.76±0.53	1.66±0.04	1.93±0.03	2.09±0.08	2.52±0.03
Plasma GtH	(ng/ml)	2.22±0.24	6.81±0.88	1.79±0.18	5.57±0.26	12.18±1.27	10.13±0.87
SR	($ng \cdot h^{-1} \cdot 25g^{-1}$)	5.84±0.37	18.79±5.03	2.97±0.25	10.75±0.67	25.46±3.63	25.53±2.50

a All data are mean ± SE.

acclimated maturing fish groups. The GSI of the two maturing fish groups did not differ significantly but were both significantly greater ($p < 0.01$) than the sexually regressed fish groups. The GSI did not differ between the 12 ± 1° C and 20 ± 1° C acclimated regressed female fish groups. The calculated value of V_i determined from sexually mature female goldfish maintained at 12 ± 1° C was significantly greater than that calculated from all other groups (Table 8). The V_i did not differ significantly among the three groups of sexually regressed fish, the average value (0.59 ml) representing about 2.4 per cent of the standard 25 g BWt. Although the calculated value of V_i from sexually maturing female goldfish acclimated to 12 ± 1° C was significantly greater ($p < 0.01$) than the 20 ± 1° C acclimated maturing females, this value was not different from any of the three groups of sexually regressed fish. The $t_{\frac{1}{2}i}$ of sexually regressed male goldfish acclimated to 20 ± 1° C was found to be significantly less ($p < 0.01$) than that calculated from both the 20 ± 1° C and 12 ± 1° C acclimated sexually regressed female groups. In contrast to the influence of the sexual state on the calculated values of $t_{\frac{1}{2}i}$ of female fish, the temperature of acclimation did not appear to have a significant influence on $t_{\frac{1}{2}i}$ from both the regressed and maturing female goldfish (Table 8). The $t_{\frac{1}{2}i}$ of sexually maturing and mature female groups (mean value 3.87 minutes) were significantly less than the $t_{\frac{1}{2}i}$ of either group of regressed female goldfish (mean value 12.25 minutes). The differences in primary MCR among the various groups did not parallel the changes in overall MCR. Thus, while the values of overall MCR

determined in sexually regressed females indicated that at 20 ± 1° C, ^{125}I-cGtH was cleared from the plasma 1.7 times faster than at 12 ± 1° C, primary MCR was increased significantly ($p < 0.01$) with the decreased acclimation temperature. Similarly, overall MCR increased significantly with the sexual state of the female fish when acclimated to 12 ± 1° C (Table 8), whereas primary MCR did not. The calculated value of primary MCR was 200 per cent greater in sexually mature female goldfish maintained at 12 ± 1° C compared to all other groups. It may be of significance that at 20 ± 1° C both the primary and overall MCR calculated from sexually maturing fish decreased significantly ($p < 0.01$) relative to the regressed female fish, perhaps contributing to the elevated plasma GtH levels observed in the former group (Table 8). Plasma GtH levels appear to be influenced by both temperature and the sexual state of the fish. Similar to overall MCR, plasma GtH levels increased significantly ($p < 0.01$) with the sexual state of the female fish acclimated to 12 ± 1° C (Table 8). In the regressed and maturing fish maintained at 20 ± 1° C, plasma GtH levels were significantly elevated over the values from 12 ± 1° C acclimated fish. The values of SR calculated from sexually regressed females acclimated to 20 ± 1° C are significantly greater ($p < 0.01$) than the values calculated from regressed male goldfish maintained at the same temperature. Pituitary SR is markedly influenced by both temperature and the sexual state of the female goldfish. SR is significantly greater at 20 ± 1° C than at 12 ± 1° C in both regressed and maturing fish and increases significantly with the sexual state of the female goldfish acclimated to either 12 ± 1° C or 20 ± 1° C.

Fig. 15. Time course of ovarian uptake of ^{125}I-cGtH in sexually regressed goldfish acclimated to either 12 ± 1° C (solid circles) (GSI = 1.70 ± 0.35%), or 20 ± 1° C (open circles) (GSI = 1.15 ± 0.07%). Data are total radioactive counts per g ovary expressed as a per cent of the total radioactive counts in the injected dose. Each sample point is the mean of duplicate samples from 5 fish ± SE.

Results of Duncan multiple range test:

$p < 0.05$

12° C	30	10	5	20	120	15	75
20° C	10	5	75	30	20	15	120

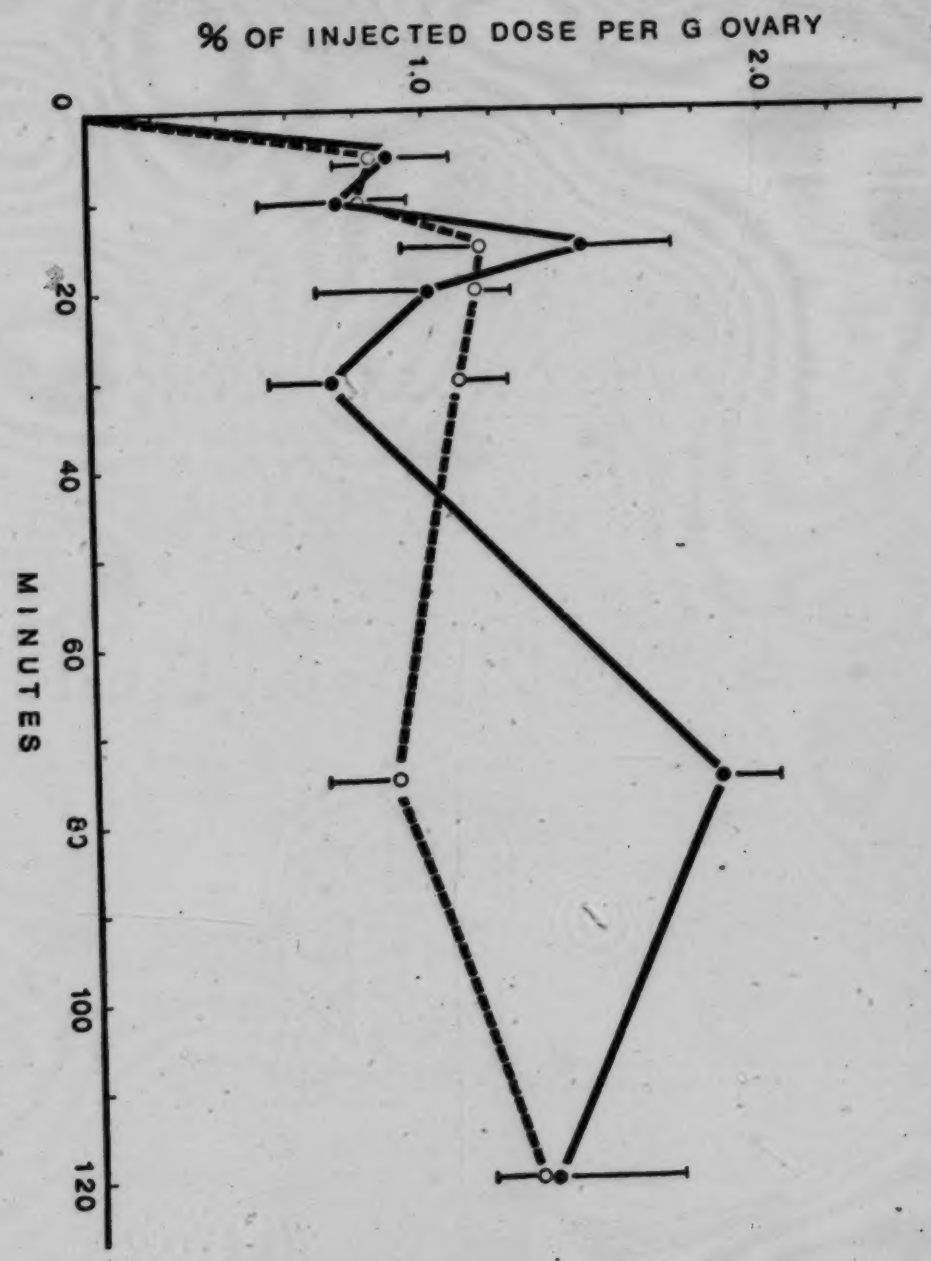
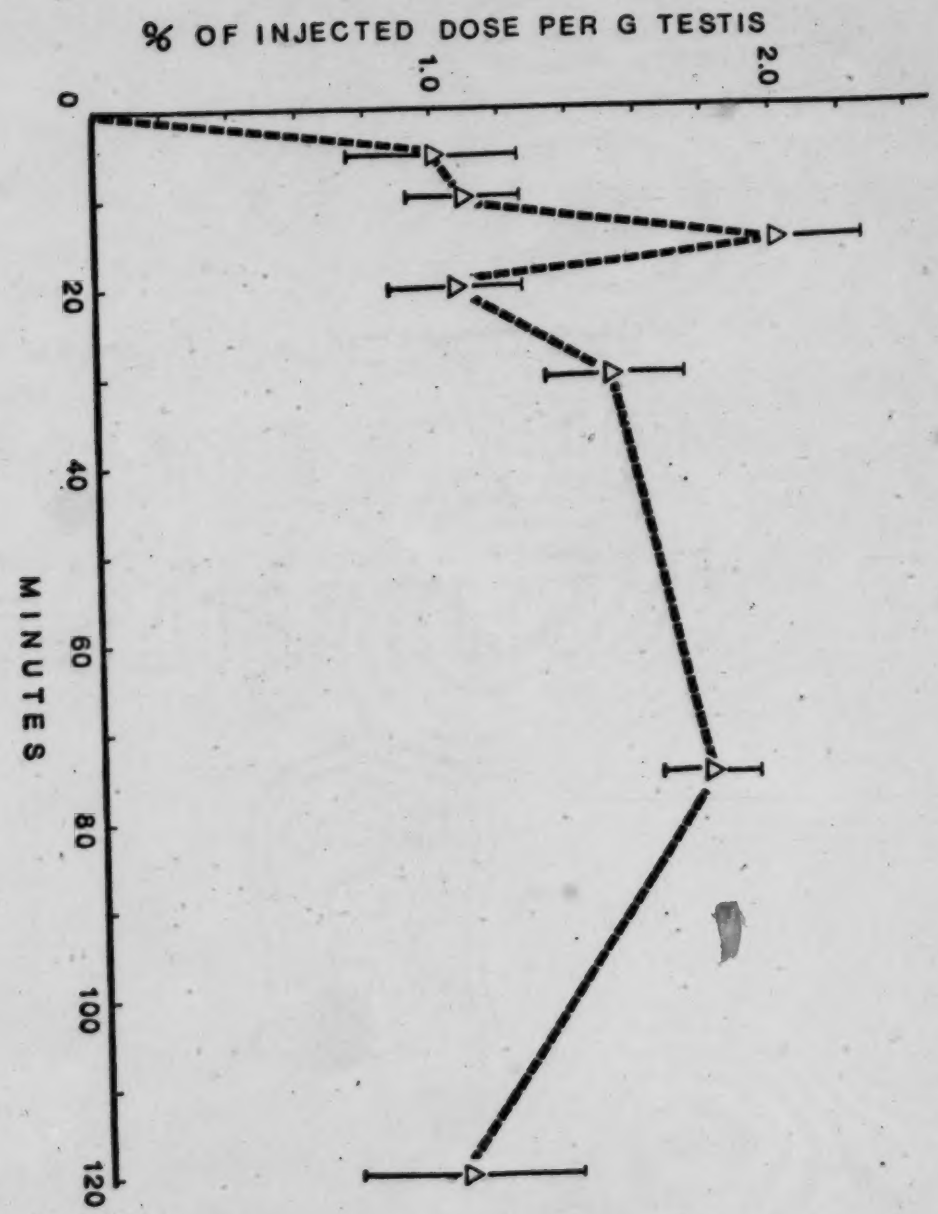

% OF INJECTED DOSE PER G OVARY
1.0
2.0
0
20
40
60
80
100
120
MINUTES

Fig. 16. Time course of testicular uptake of ^{125}I-cGtH in sexually regressed male goldfish (GSI = 0.75 ± 0.10%) maintained at 20 ± 1° C. Data are total radioactive counts per g testis expressed as a per cent of the total radioactive counts of the injected dose. Each sample point is the mean of individual samples from 5 fish ± SE.

Results of Duncan multiple range test:

$p < 0.05$

12° C	5	120	20	10	30	75	15


% OF INJECTED DOSE PER G TESTIS
1.0
2.0
0
20
40
60
80
100
120
MINUTES

Kinetics of gonadal uptake of glycoprotein ^{125}I-cGtH

Figure 15 illustrates the time course of uptake of ^{125}I-cGtH by the ovaries of sexually regressed goldfish. Although the large amount of variability between individual fish precludes a definitive analysis, it appears that a plateau in ovarian uptake is attained during the first 15 minutes after the intraarterial injection of ^{125}I-cGtH. The maximum ovarian specific activity occurs at 75 and 120 minutes post-injection in cold and warm acclimated regressed goldfish, respectively. There are no major differences in the overall pattern of gonadal uptake due to acclimation temperature in regressed females, although at 75 minutes post-injection the fish maintained at 12 ± 1° C have a significantly greater ovarian specific activity compared to the fish at 20 ± 1° C. Averaged over the 120 minute sampling period, the value of ovarian specific activity for both experiments is 1.10 ± 0.18 per cent of the injected dose per g ovary. This value is equivalent to only 0.29 per cent of the injected dose per total gonad of a standard 25 g regressed female fish. It is important to note that the total radioactivity counted from each ovarian sample will include the ^{125}I-cGtH specifically bound to ovarian receptors, in addition to non-specifically adsorbed and circulating ^{125}I-cGtH, and the radio-iodinated degradation products.

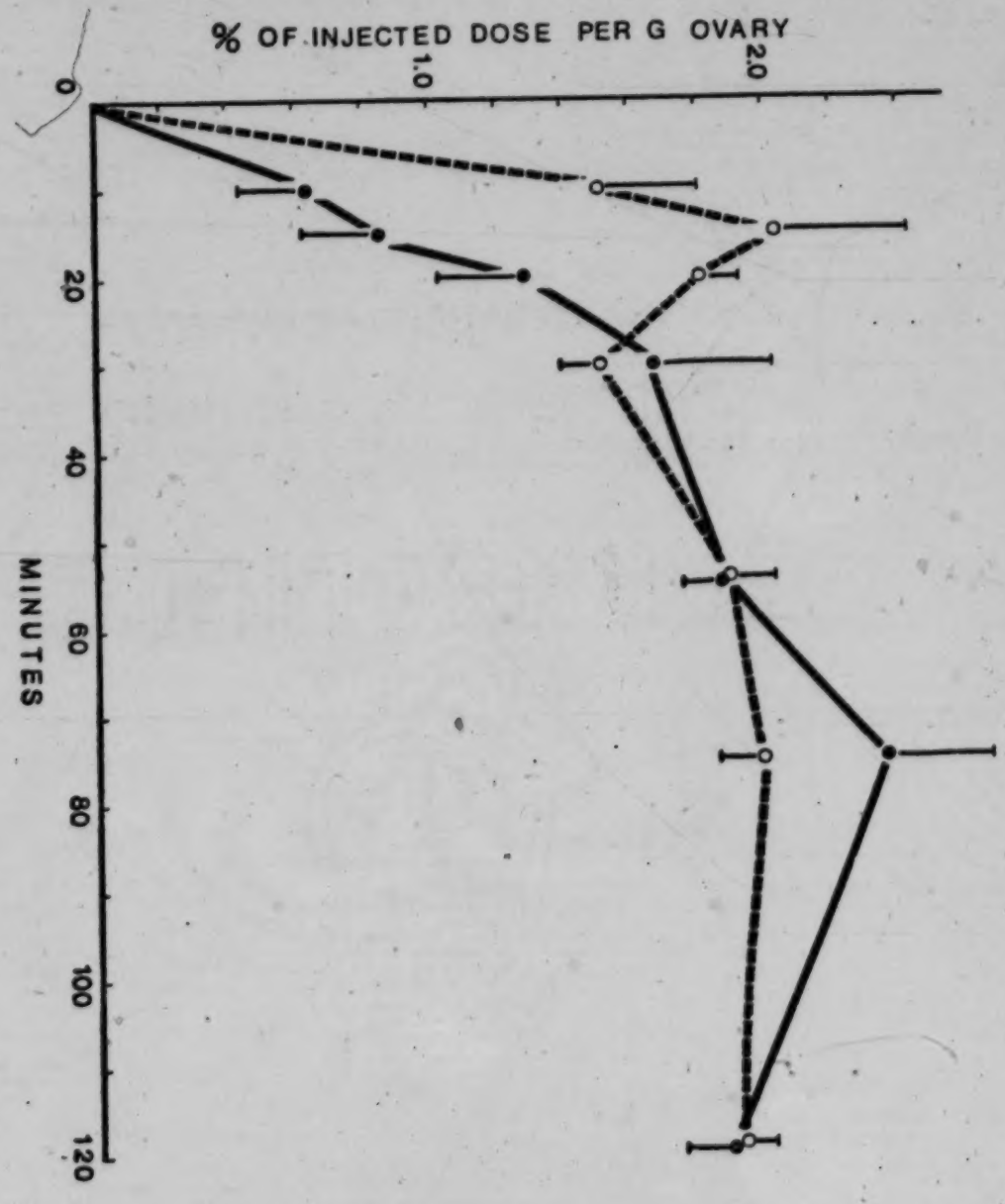
The time course of testicular uptake of intraarterially injected ^{125}I-cGtH in goldfish acclimated to 20 ± 1° C is shown in Figure 16. The maximum specific activity of the testis is attained

Fig. 17. Time course of ovarian uptake of ^{125}I-cGtH by goldfish undergoing ovarian recrudescence acclimated to either 12 ± 1° C (solid circles) (GSI = 4.74 ± 0.45%) or 20 ± 1° C (open circles) (GSI = 5.02 ± 0.35%). Data are total radioactive counts per g ovary expressed as a per cent of the total radioactive counts in the injected dose. Each sample point is the mean of duplicate samples from 5 fish ± SE.

Results of Duncan multiple range test:

$p < 0.05$

12° C	10	15	20	30	55	75	120
20° C	30	10	20	55	120	75	15


% OF INJECTED DOSE PER G OVARY
0
1.0
2.0
MINUTES
0
20
40
60
80
100
120

15 minutes post-injection, followed by a significant ($p < 0.05$) decline to the 20 minute mean value. The value of specific activities averaged over the 120 minute sampling period is 1.39 ± 0.16 per cent of the injected dose per g testis and is not significantly different from that obtained in the regressed females. This represents about 0.20 per cent of the total injected dose per gonad of the standard 25 g regressed male goldfish. The general pattern of gonadal uptake observed in sexually regressed males (Figure 16) is similar to that seen in regressed females at the same temperature (Figure 15). The testicular specific activity at 75 minutes post-injection is, however, significantly elevated ($p < 0.01$) over the corresponding ovarian mean value.

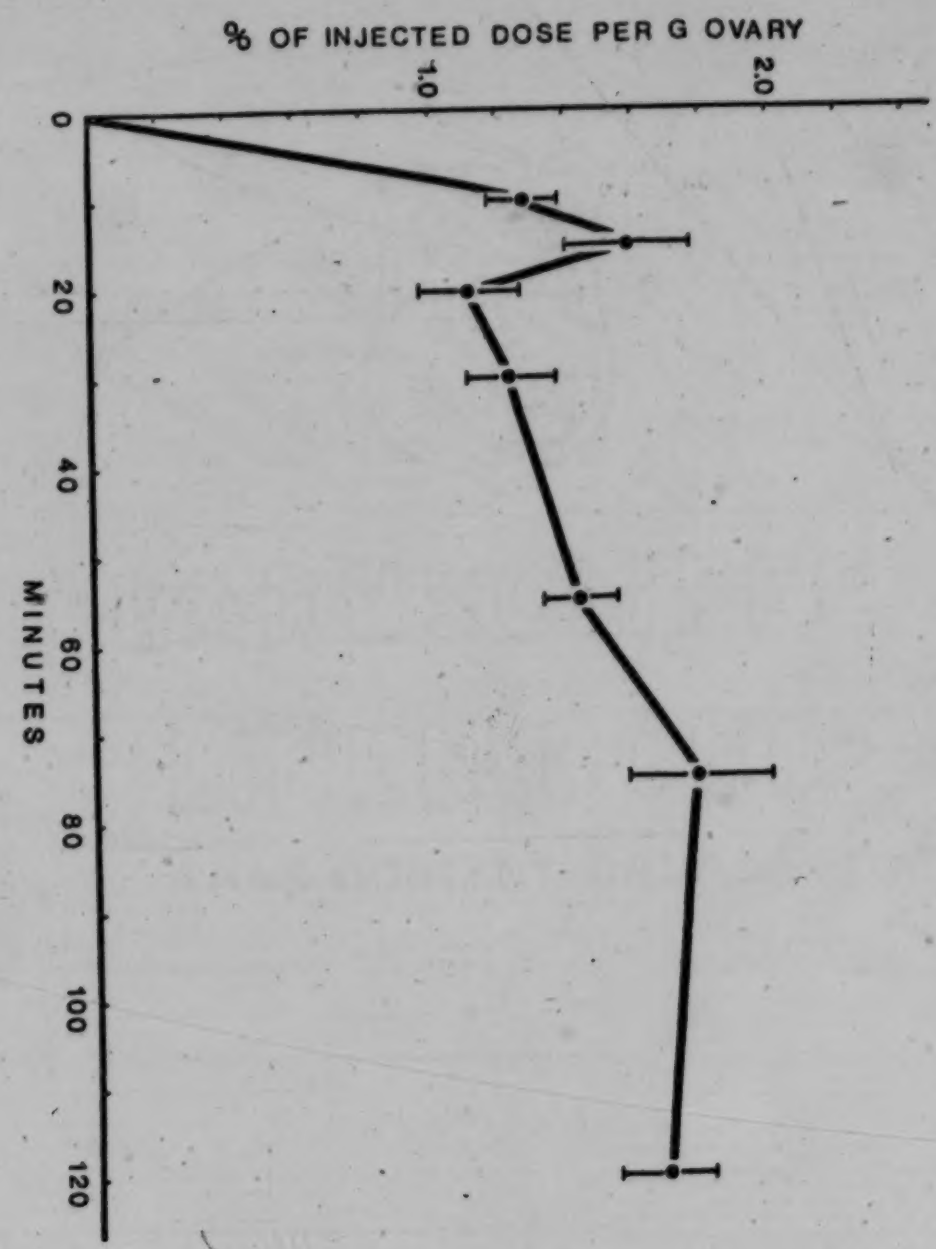
Figure 17 shows the time course of uptake of ^{125}I-cGtH by the ovaries of sexually maturing goldfish maintained at 12 ± 1° C and 20 ± 1° C. Maturing fish acclimated to 20 ± 1° C exhibit a very rapid increase in ovarian specific activity, reaching the maximum value (2.04 ± 0.37 per cent of injected dose per g ovary) at 15 minutes post-injection (Figure 17). After 15 minutes the level of ovarian specific activity remains relatively unchanged, although the value at 30 minutes is significantly less ($p < 0.05$) than the value at 15 minutes. Ovarian specific activity of fish maintained at 20 ± 1° C is significantly elevated ($p < 0.01$) over the values from cold acclimated fish at 10, 15 and 20 minutes post-injection, indicating that the rate of uptake is faster at 20 ± 1° C than at 12 ± 1° C. The maximum level of ovarian specific activity was not attained until 75 minutes post-injection in maturing fish

Fig. 18. Time course of ovarian uptake of ^{125}I-cGtH by sexually mature goldfish (GSI = 15.41 ± 0.64%) acclimated to 12 ± 1° C. Data are total radioactive counts per g ovary expressed as a per cent of the total radioactive counts in the injected dose. Each sample point is the mean of duplicate samples from 5 fish ± SE.

Results of Duncan multiple range test:

$p < 0.05$

12° C	20	30	10	55	120	15	75


% OF INJECTED DOSE PER G OVARY
1.0
2.0
0
20
40
60
80
100
120
MINUTES

maintained at 12 ± 1° C (Figure 17). The plateau level of ovarian radioactivity was not statistically different between the two groups of fish, the average value being 1.88 ± 0.16 per cent of the injected dose per g ovary. This value represents about 2.29 per cent of the total injected dose per gonad of a standard 25 g sexually maturing female fish, and is considerably greater than the same value calculated from sexually regressed fish.

The time course of uptake of ^{125}I-cGtH by the ovaries of sexually mature goldfish (GSI = 15.41 ± 0.64%) acclimated to 12 ± 1° C is shown in Figure 18. In contrast to sexually maturing goldfish acclimated to the same temperature, ovarian specific activity is significantly elevated to near maximal levels as early as 15 minutes post-injection. Comparison with goldfish undergoing ovarian recrudescence suggests that, although the rate of uptake during the first 15 minutes post-injection is increased, the specific activity is significantly less in sexually mature fish (1.60 ± 0.07 per cent of the injected dose per g ovary). This value, however, represents about 6.16 per cent of the total injected dose per gonad of a standard 25 g sexually mature female goldfish, and is much greater than the same value calculated from both regressed and maturing fish.

Distribution of ^{125}I-cGtH

The distribution of TCA-precipitated radioactivity in sexually regressed male and female goldfish tissues and plasma, 15 minutes after the intraarterial injection of ^{125}I-cGtH is shown in Tables 9 and 10. The greatest concentration of tissue specific activity

TABLE 9. Tissue and plasma distribution of TCA-precipitated ^{125}I-cGtH, 15 minutes after intraarterial injection in sexually regressed female and male goldfish (GSI = 2.84 ± 0.14% and 1.97 ± 0.48%, respectively), acclimated to 12 ± 1° C.

Tissue	Per Cent of Injected Dose per g Tissue	
	female	male
kidney	98.31 ± 6.11[a]	80.01 ± 10.06
spleen	24.99 ± 2.10	30.25 ± 3.52
heart	11.50 ± 2.04	10.01 ± 1.92
hypothalamus	8.34 ± 2.34	6.02 ± 1.53
gall bladder[b]	7.89 ± 3.32	3.03 ± 1.09
air bladder	4.31 ± 0.34	3.66 ± 0.44
gills	4.30 ± 0.52	4.46 ± 0.51
liver	3.56 ± 0.33	3.54 ± 0.49
intestine	3.12 ± 0.35	2.73 ± 0.48
eyes	2.72 ± 0.42	1.93 ± 0.11
oral fat pad	2.73 ± 0.48	2.54 ± 0.27
brain[c]	2.26 ± 0.42	1.25 ± 0.15
ovary	1.60 ± 0.11	---
testis	---	1.40 ± 0.32
muscle	1.43 ± 0.20 (4)	1.35 ± 0.17
Plasma (Per Cent of Injected Dose per ml)	43.44 ± 3.63	44.17 ± 3.98

a All data are mean ± SE; N = 5 except where noted.

b Gall bladder includes bile fluid.

c The brain tissue includes all of the brain except lateral and medial lobes of the hypothalamus which were pooled for ^{125}I-cGtH uptake assay.

TABLE 10. Tissue and plasma distribution of TCA-precipitated ^{125}I-cGtH, 15 minutes after intraarterial injection in sexually regressed female and male goldfish (GSI = 2.84 ± 0.14% and 1.97 ± 0.48%, respectively), acclimated to 12 ± 1° C.

Tissue	Per Cent of Injected Dose per Total Organ Weight	
	female	male
muscle[a]	28.60 ± 4.00[b](4)	27.00 ± 3.40
kidney	20.64 ± 1.28	16.80 ± 2.11
intestine	4.52 ± 0.51	3.96 ± 0.70
gills	3.65 ± 0.44	3.79 ± 0.43
spleen	2.75 ± 0.23	3.32 ± 0.39
liver	2.60 ± 0.24	2.58 ± 0.36
oral fat pad	1.36 ± 0.24	1.27 ± 0.13
ovary	1.14 ± 0.08	---
testis	---	0.63 ± 0.14
eyes	0.87 ± 0.13	0.62 ± 0.03
heart	0.63 ± 0.11	0.55 ± 0.10
gall bladder[c]	0.63 ± 0.26	0.24 ± 0.09
air bladder	0.47 ± 0.04	0.40 ± 0.05
brain[d]	0.23 ± 0.04	0.12 ± 0.02
hypothalamus	0.17 ± 0.05	0.12 ± 0.03
Plasma[e] (Per Cent of Injected Dose per 0.75 ml)	32.58 ± 2.72	33.12 ± 2.98

a Total muscle weight calculated as weight of all organs and plasma subtracted from total BWt. This weight would include bone weight.

b All data are mean ± SE; N = 5 except where noted.

c Gall bladder includes bile fluid.

d The brain tissue includes all of the brain except lateral and medial lobes of the hypothalamus which were pooled for ^{125}I-cGtH uptake assay.

e Total plasma volume estimated as 3 per cent of standard 25 g BWt.

is found in the kidney (Table 9), where it is more than double that found in the plasma of female goldfish. Apart from the kidneys, the tissues of female goldfish with concentrations of ^{125}I-cGtH greater than in the body as a whole were spleen, heart, hypothalamus, gall bladder, air bladder and gills. In males the kidney, spleen, heart, hypothalamus and gills concentrated more TCA-precipitated radioactivity than the body as a whole (Table 9). It is perhaps surprising to note the relatively minor contribution, both in terms of ^{125}I-cGtH tissue specific activity and organ accumulation, of the gonads in sexually regressed male and female goldfish. However, since gonadotropin is likely to exert its action on the cell plasma membrane (Cuatrecases, 1974; Sutherland, 1972), expressing the data per unit weight could bias the results in tissues such as the ovary and testes which have either a relatively small or changing surface area to weight ratio (A.F. Cook, unpublished observations). Although tissue specific activity may estimate the tissue metabolism of ^{125}I-cGtH (Table 9), adjustments for the total organ or tissue weight must be made to assess the contribution of a particular organ or tissue to the distribution of ^{125}I-cGtH in the fish (Table 10). Table 10 shows that muscle (which has the lowest tissue specific activity; Table 9) provides a major site of accumulation of TCA-precipitated radioactivity 15 minutes after injection. In contrast, the hypothalamus, which has a relatively high specific activity (Table 9) plays only a very minor role in the total distribution of ^{125}I-cGtH (Table 10).

DISCUSSION

The results obtained from Part I of the present study (Intraperitoneal Injections of cGtH) can contribute to our knowledge of the reproductive endocrinology of teleost fishes in a number of ways. Although a large number of studies have utilized the intraperitoneal site for the injection of GtH (Billard and Escaffre, 1973; Nayyar *et al.*, 1976; Upadhyay, 1977; De Montalembert *et al.*, 1978; Gordon and Zohar, 1978; Jalabert *et al.*, 1978; R.E. Peter, unpublished results; Stacey *et al.*, 1979b), a lack of information concerning post-injection circulating GtH levels has limited their interpretation. We have recently described the relationship between temperature and the latent time to ovulation after an intraperitoneal injection of human chorionic gonadotropin (HCG) in goldfish (Stacey *et al.*, 1979b). The present study suggests that the increase in latency to ovulation at lower temperatures may, in part, represent delayed uptake of HCG from the peritoneal cavity to the circulatory system. Also, it has been shown that intraperitoneal injection of cGtH, at a dose similar to that used in the present study, is more effective in causing ovarian development in goldfish acclimated to 13 to 14° C at one time in the daily photoperiod than at other times (R.E. Peter, unpublished results). The present results indicate that the injected cGtH will nearly all be cleared from the blood within 24 hr. Further, a knowledge of the plasma disappearance profile and MCR of GtH after an intraperitoneal injection may contribute to the determination of the most appropriate

time and temperature at which to administer GtH to effect ovulation and spawning in a number of commercially important teleost species. It is possible that the poor success in the artificial spawning of a number of carp species (Chaudhuri, 1976; Hora, 1945) may in part, be attributed to a failure to appreciate both the rapid uptake of GtH from the peritoneal cavity into the circulatory system and the rapid plasma GtH clearance after intraperitoneal administration at elevated temperatures.

In clearance studies based on a single injection of tracer hormone, it is essential that the tracer enter the vascular system as a bolus. The results obtained from the intraperitoneal injection experiments indicate an undesirable effect of delayed hormone uptake from the peritoneal cavity to the circulatory system on both clearance and distribution parameters. The calculated values of V_i after intraperitoneal injection represents a volume of approximately 50 per cent of the total BWt of the standard 25 g goldfish. These values are much too large to represent the true vascular volume in the goldfish. In mammals, a number of clearance studies on GtH have demonstrated V_i to represent from 2 to 7 per cent BWt (Bogdanove and Gay, 1969; Odell *et al.*, 1967; Weick, 1977). Similar V_i values of 2 to 5 per cent BWt have been determined for goldfish after intra-arterial injection of ^{125}I-cGtH (Table 8). In addition, the estimated $t_{\frac{1}{2}}$ (ca. 8.5 hr) for cGtH after an intraperitoneal injection is much greater than that estimated from the rate of decline in serum GtH levels following the ovulatory GtH surge in intact goldfish (Stacey *et al.*, 1979a; APPENDIX IV) or that determined after an

intraarterial injection (present study). It is probable that the apparent lack of effect of temperature on plasma MCR after an intraperitoneal injection of cGtH (Table 1) is a consequence of an increased rate of uptake into the blood, balanced against a higher MCR at 20 ± 1° C, compared to 12 ± 1° C. The difference in the time course of uptake, as indicated by the difference in timing of the peak levels of serum GtH following intraperitoneal injection (see Figures 1 and 2), supports this possibility. The necessity of direct intraarterial injection of tracer GtH for the study of MCR is, then, obvious.

The validity of the results obtained from the intraarterial injection of ^{125}I-cGtH is based upon three major assumptions. The first assumption is that the immunoprecipitation system used in the present study provides a quantitative measurement of biologically active GtH. The immunoprecipitation of ^{125}I-cGtH in goldfish plasma was developed to avoid counting free radioiodine or small fragments of radioiodinated GtH, and including these as if they were intact GtH molecules in the determination of GtH clearance and distribution values. Comparison of the results obtained with the immunoprecipitation method, to those determined using TCA-precipitated or total radioactivity, demonstrates that under certain physiological conditions the contribution of non-immunoreactive radioiodinated fragments can markedly alter the calculated clearance parameters (Tables 2, 3 and 7). However, it is possible that the immunoprecipitation system may measure radioiodinated GtH that does not possess full biological activity. The best evidence that the antisera

used in the present study does provide quantitative measurements of biologically active GtH are the results of our recent study (Stacey *et al.*, 1979a; APPENDIX IV) in which the periovulatory pattern of immunoreactive serum GtH levels of sexually mature female goldfish are described. Also, a number of studies have shown that carp (Breton and Weil, 1973; Weil *et al.*, 1975) and goldfish (Crim *et al.*, 1976) respond to injections of LH-FSH/RH (luteinizing hormone-follicle stimulating hormone/releasing hormone) by an elevation in circulating immunoreactive GtH levels. Since LH-FSH/RH can induce germinal vesicle migration and breakdown in carp (Sokolowska *et al.*, 1978) and ovulation in the goldfish (Lam *et al.*, 1975; Lam *et al.*, 1976), it is probable that the GtH RIA and the immunoprecipitation technique employed in the present study provide quantitative measurements of biologically active GtH.

The second assumption is that the glycoprotein ^{125}I-cGtH employed in the present study is indistinguishable from endogenous goldfish GtH in terms of clearance and distribution. A number of findings herein indicate that the ^{125}I-cGtH is not treated as a foreign protein by the fish, and that it has biological activity, which supports the validity of this assumption. First, the results of PART II of the present study demonstrate major differences in the plasma clearance and ovarian uptake of ^{125}I-cGtH compared to a similarly radioiodinated foreign protein, ^{125}I-BSA. The prolonged plasma clearance of the foreign tracer ^{125}I-BSA relative to the labelled cGtH (Figure 6) is in agreement with the work of de Kretser *et al.* (1973). Their data demonstrate greatly increased values of

$t_{\frac{1}{2}_i}$ for ^{131}I-ovine luteinizing hormone in rams when the tracer preparation was not purified by both gel filtration and ion exchange chromatography, or stored for extended periods prior to injection, suggesting that impure or 'damaged' iodinated hormone is characterized by a prolonged plasma $t_{\frac{1}{2}_i}$. In addition, the *in vivo* competitive uptake experiments demonstrate that much of the glycoprotein ^{125}I-cGtH was bound at physiologic ovarian receptors, rather than adsorbed nonspecifically to the ovary of sexually maturing female goldfish. Also, the finding that the sexual state of the female goldfish influences the amount of ovarian uptake of ^{125}I-cGtH suggests that the radioiodinated hormone retains biological activity. Further, we have demonstrated (A.F. Cook, G.A. Longmore and R.E. Peter, unpublished observations) autoradiographic localization of ^{125}I-cGtH in specific follicle cells of the maturing goldfish ovary. The topographic location of some of the heavily labelled cells in the thecal layer around oocytes is similar to that described for the presumed steroidogenic 'special thecal cells' of the preovulatory follicle in the goldfish (Nagahama *et al.*, 1976; Hoar and Nagahama, 1978). Also, it has been shown that a number of mammalian gonadotropins retain significant amounts of their original biological activity when iodinated by methods similar to that employed in the present study (human chorionic gonadotropin: Midgely, 1966; Lunenfeld and Eshkol, 1967; Leidenberger and Reichert, 1972; luteinizing hormone: Kohler *et al.*, 1968; Kammerman *et al.*, 1972; Danzo *et al.*, 1972). It would appear then that the radioiodinated cGtH employed in the present study is a valid tracer to gain informa-

tion concerning the clearance and distribution of endogenous goldfish GtH.

The third major assumption, that a steady-state of GtH secretion existed during the course of each clearance study (see APPENDIX II), is supported by the recent study of Hontela and Peter (1978). These workers described the influence of temperature, photoperiod and sexual condition on the daily variations in serum GtH levels in the female goldfish. In contrast to goldfish with a regressed gonad, sexually maturing and mature female goldfish had significant daily variations in serum GtH levels (Hontela and Peter, 1978). Their data indicate that during the period of the photophase in which the present clearance studies were performed (0900 - 1400 hr), serum GtH levels are either constant or show variations of small magnitude relative to other periods of the daily pattern. Also, none of the experiments were done on fish undergoing ovulation, which is known to be caused by a large surge of GtH (Stacey *et al.*, 1979a; APPENDIX IV), and may entail changes in the kinetics of GtH.

The results obtained in the present study describe for the first time in a teleost fish some aspects of the dynamics of the physiology of GtH during an annual reproductive cycle. With the reservations discussed above, the importance of the study is twofold. First, it permits an assessment of previous studies based on circulating GtH levels, and may therefore contribute to our knowledge of the mechanisms regulating teleost reproduction. Secondly, not only is a knowledge of the GtH clearance and distribution parameters in an ectothermic vertebrate of interest for its own sake, but comparison

with similar studies on some mammalian species (see below) may provide a valuable contribution to the overall understanding of hormone clearance.

The overall plasma MCR of ^{125}I-cGtH varied during the three stages of the annual reproductive cycle of female goldfish. At 12 ± 1° C, overall MCR increased with the increasing state of ovarian development (Table 8), so that in March, sexually mature female goldfish show a 150 per cent increase in the overall MCR compared to sexually regressed female goldfish in August and September. At 20 ± 1° C, however, overall MCR does not appear to vary appreciably between sexually regressed and maturing female goldfish. The finding that overall MCR is greater at 20 ± 1° C than at 12 ± 1° C in sexually regressed fish, but not in sexually maturing fish, emphasizes that both temperature and the sexual state of development can influence the overall plasma MCR of GtH.

There is no clear trend in the calculated values of primary MCR in relation to either sex, state of ovarian development or temperature. The primary MCR determined in sexually regressed male goldfish maintained at 20 ± 1° C is less than that of sexually regressed females acclimated to either 20 ± 1° C or 12 ± 1° C. Values of primary MCR of both groups of regressed female goldfish were similar to those of 12 ± 1° C acclimated maturing females and were greater than the maturing females maintained at 20 ± 1° C. The sexually mature females maintained at 12 ± 1° C showed a twofold increase in primary MCR compared to all other groups. The marked increase in the primary plasma MCR of the sexually mature females

may be related to their increased value of V_i (Table 8). An increase in the rate of transfer of the labelled hormone from the vascular pool into the tissue spaces could contribute to the observed elevated values of primary MCR and V_i in these fish. The possible physiological implications of this result are not known.

Plasma GtH levels also show marked differences in response to the temperature of acclimation and reproductive state. The plasma GtH level of sexually regressed females maintained at 20 ± 1° C is nearly four times greater than that of 12 ± 1° C acclimated regressed female goldfish. Similarly, there is a marked increase in plasma GtH levels of 20 ± 1° C acclimated maturing goldfish compared to maturing females maintained at 12 ± 1° C. At both 12 ± 1° C and 20 ± 1° C the plasma GtH levels increase through the three stages of the annual reproductive cycle of female goldfish examined in the present study. These data are similar to results reported by other workers (Hontela and Peter, 1978; Gillet *et al.*, 1977, 1978), which also demonstrate an influence of both temperature and state of gonadal development on circulating GtH levels. Since overall MCR changes during ovarian development and with temperature, it is clear that measurement of static GtH concentrations in the serum or plasma provide only a limited assessment of the GtH dynamics in a particular physiologic situation.

The calculated values of V_i vary between 2 and 5 per cent of the standard 25 g BWt goldfish in the present intraarterial injection experiments. The largest V_i was found in sexually mature female goldfish acclimated to 12 ± 1° C (Table 8). These values for

V_i in the goldfish are similar to those reported from a number of clearance studies done on mammals (see above).

The calculated values of $t_{\frac{1}{2}_i}$ are unaffected by the temperature of acclimation in both regressed and sexually maturing female goldfish. The finding that the $t_{\frac{1}{2}_i}$ values are substantially greater in sexually regressed female goldfish compared to both maturing and mature fish suggests that GtH may have more physiological importance in the latter two groups. A number of studies have demonstrated that the calculated values of $t_{\frac{1}{2}_i}$ increase with decreasing biological activity of the hormone (de Kretser, 1973), and, in the present study, the foreign protein ^{125}I-BSA was found to be cleared much more slowly than the ^{125}I-cGtH. It is possible that some alteration in the metabolic clearance mechanism of glycoprotein GtH occurs at a stage early in ovarian recrudescence, and is responsible for the observed decline in calculated values of $t_{\frac{1}{2}_i}$. It may also be relevant to note that teleost glycoprotein GtH's, although apparently devoid of biological activity in the process of vitellogenin uptake, are very potent in final oocyte maturation (Ng and Idler 1978a,b and see INTRODUCTION). However, since a salmon glycoprotein GtH is capable of inducing oocyte maturation (Jalabert *et al.*, 1974), as well as stimulating cyclic adenosine monophosphate activity in the ovary (Idler *et al.*, 1975; D.R. Idler, personal communication) and ^{33}P-incorporation into chick testes (Idler *et al.*, 1975), it is probable that the teleost glycoprotein GtH's are involved in a variety of reproductive processes. The results reported by Hontela and Peter (1978) also

demonstrate differences in the blood levels of glycoprotein GtH between sexually regressed female goldfish and both maturing and mature fish. Their findings indicated that sexually regressed goldfish either did not show significant daily variations in serum immunoreactive GtH levels under different environmental regimes, or showed daily fluctuations considerably smaller in magnitude than those of females undergoing ovarian recrudescence or mature females (Hontela and Peter, 1978; Peter and Hontela, 1978). It would appear that a number of aspects of the glycoprotein GtH dynamics are markedly different between sexually regressed, and both maturing and mature female goldfish.

The finding that the overall MCR and $t_{\frac{1}{2}i}$ can change during the annual reproductive cycle of the female goldfish is in contrast to the results reported in a number of mammalian clearance studies. For example, Akbar *et al.* (1974) demonstrated relatively constant rates of plasma metabolic clearance for ovine luteinizing hormone (oLH) and ovine follicle stimulating hormone (oFSH) in ewes during the mid-luteal and follicular phases of the estrous cycle, and on the day of estrus. Also, the plasma MCR of rat prolactin (Koch *et al.*, 1971), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Eder and Lipner, 1970; Bogdanove and Gay, 1969) are not influenced by the estrous cycle. Limited data also suggests that the plasma MCR of human chorionic gonadotropin (HCG) (Rizkallah *et al.*, 1969), human LH (Kohler *et al.*, 1968) and human FSH (Coble *et al.*, 1969) do not differ during a number of different physiological states.

The results of the present study provide the first quantitative evidence suggesting that the pituitary gland SR of GtH changes throughout an annual reproductive cycle in a teleost fish. The pituitary SR in sexually mature females maintained at 12 ± 1° C is higher than in recrudescing or regressed females acclimated to the same temperature, and is equivalent to the calculated SR for recrudescing females at 20 ± 1° C (Table 8). In addition, the effect of temperature on plasma GtH levels (see above) may be attributed to increased secretion rate by the pituitary gland (Table 8). Although the influence of temperature on the calculated value of SR is more pronounced in sexually regressed fish than in female fish undergoing ovarian recrudescence, the absolute levels of SR are considerably greater in the latter group (Table 8).

A number of studies have demonstrated that circulating GtH levels gradually increase as the gonads progressively mature, from a low GtH level in immature fish to higher values in mature fish (Crim *et al.*, 1975; Breton *et al.*, 1975; Breton *et al.*, 1977) with a marked and transient elevation of circulating GtH at ovulation and spermiation (Breton *et al.*, 1972; Crim *et al.*, 1975; Stacey *et al.*, 1979a, see APPENDIX IV). This has led to the hypothesis that incremental blood levels of GtH are required to stimulate progressive development of the gonads (see Peter and Crim, 1979; Peter and Hontela, 1978). The results of Hontela and Peter (1978) suggest that daily fluctuations, or surges, in blood levels of GtH are also of importance in stimulating gonadal development. The present results indicate that the changes in blood levels of GtH seen during progressive

gonadal development are a reflection of progressive increases in pituitary SR of GtH.

A number of studies have speculated that the elevated plasma or serum GtH levels determined in recrudescing fish exposed to warm temperatures are a result of an increased rate of pituitary GtH secretion, and are stimulatory to gonadal recrudescence (Gillet *et al.*, 1977; Gillet *et al.*, 1978). The present study provides quantitative information supporting the hypothesis of the stimulatory effect of warm temperature on GtH secretion. However, in the present study, the GSI of the maturing fish group acclimated to 20 ± 1° C for 14 days was not greater than the GSI of the cold acclimated maturing female goldfish group. It is possible that the observed increase in pituitary SR of the warm acclimated females is not reflected by an increased GSI because of the relatively brief exposure to the stimulatory temperature.

The rate of ovarian uptake of ^{125}I-cGtH in 12 ± 1° C acclimated sexually maturing female goldfish was considerably less than that of the maturing females maintained at 20 ± 1° C. Although the plateau level of ovarian specific activity attained did not differ in these two groups, it was considerably greater than that of any of the groups of sexually regressed fish. There were no major differences in the overall pattern of uptake between the sexually regressed female fish acclimated to either 12 ± 1° C or 20 ± 1° C, or to regressed males maintained at 20 ± 1° C. The rate of uptake by the ovaries of sexually mature fish maintained at 12 ± 1° C was greater than that of either the maturing or regressed fish, although the

plateau level of ovarian radioactivity was less than that of both groups of sexually maturing female goldfish. The finding that the time course of gonadal uptake of glycoprotein ^{125}I-cGtH varies depending on the state of sexual development and temperature may clarify a number of findings outlined above. The absence of a difference between either the rate of uptake or the plateau levels of ovarian specific activity in sexually regressed female goldfish maintained at 12 ± 1° C and 20 ± 1° C may be responsible for the lack of effect on GSI of the elevated SR and plasma GtH levels in the latter group. It is possible that the relatively low levels of radioactivity accumulated in the gonad of the sexually regressed female fish may reflect a state of unresponsiveness to circulating GtH.

The effect of temperature on the time course of uptake of ^{125}I-cGtH was more pronounced in sexually maturing female goldfish (Figure 17) than in fish with a regressed gonad (Figure 15). At 20 ± 1° C the rate of ovarian ^{125}I-cGtH uptake by sexually maturing goldfish, during the first 20 minutes post-injection was much greater than that of 12 ± 1° C acclimated fish. This may have some causal effect in inducing more rapid gonadal recrudescence at warm temperatures, although the maximum level of radioactivity incorporated into the ovary did not differ between the two groups. From these results, and effects on pituitary SR and plasma GtH levels outlined above, it can be hypothesized that temperature may influence ovarian recrudescence in the goldfish in at least two ways. Rising water temperatures in the spring could increase the pituitary SR and blood GtH levels,

causing an increased metabolic response by the ovary and/or the response of the ovary to the circulating GtH levels may be directly affected by elevated temperature. Whatever the mechanism, it is clear that temperature plays a very important role in GtH physiology and ovarian recrudescence in the goldfish.

The finding that sexually maturing fish exhibit greater ovarian incorporation of glycoprotein ^{125}I-cGtH than the regressed fish at both 12 ± 1° C and 20 ± 1° C (Figure 17) may in part be reflected by the decreased $t_{\frac{1}{2}i}$ values for GtH in the maturing fish. It is interesting to speculate that in the sexually regressed fish the ovary is relatively unresponsive to the circulating glycoprotein GtH which in some manner contributes to the observed elevated values of $t_{\frac{1}{2}i}$ (Table 8), but after some stage early in the process of recrudescence, either the number or affinity of ovarian receptors increases concomitant with decreased values of $t_{\frac{1}{2}i}$. Perhaps such an alteration in the response of the ovary to circulating GtH may also be related to the onset of a positive response of the pituitary-gonadal axis to an elevated temperature.

The finding that the ovaries of sexually mature fish maintained at 12 ± 1° C show a more rapid initial uptake of glycoprotein ^{125}I-cGtH (Figure 18) than found in sexually maturing female goldfish acclimated to the same temperature suggests also that the capacity of the ovary to bind circulating GtH increases as development of the ovary proceeds. The significant decrease in the plateau level of ovarian specific activity of the sexually mature fish relative to the maturing fish is likely related to the large decrease in the

ratio of surface area to volume of the ovary of sexually mature fish and the increase in yolk content of the mature ovary. It is obvious much work is required to directly address this important problem of the relationship of ovarian GtH binding and the annual reproductive cycle.

TCA-precipitated radioactivity was found in all the major tissues of the goldfish 15 minutes after an intraarterial injection of ^{125}I-cGtH (Tables 9 and 10). The finding that the kidney was the major site of accumulation (98 per cent of injected dose per g kidney; Table 9) provides the first evidence in teleosts that uptake of GtH by non-target tissue may play an important role in regulating circulating hormone levels and may contribute to the short circulatory $t_{\frac{1}{2}_i}$. Large increases in the half-life of circulating LH occur following nephrectomy of sheep (de Kretser *et al.*, 1973) or castrated male rats (Gay, 1974). A number of mammalian studies have also shown that the kidney has a large capacity to accumulate and catabolize LH (Butt *et al.*, 1974; Braunstein *et al.*, 1972; Ascoli *et al.*, 1976; de Kretser *et al.*, 1973). It should be noted, however, that the principle mechanism by which ^{3}H-oLH is removed from the circulatory system of the rat is by glomerular filtration (Ascoli and Puett, 1976b). These workers have also demonstrated that serum chromatograms are characterized by two biologically active, molecular weight components following an intravenous injection of ^{3}H-oLH in the rat (Ascoli and Puett, 1976a,b). A large molecular weight fraction involves non-covalent interaction with circulating proteins and is

cleared very slowly, mainly due to reduced urinary extraction (Ascoli and Puett, 1976b). A second component was co-chromatographed with ^{3}H-oLH and was cleared similar to the injected ^{3}H-oLH. These authors suggest that the high molecular weight fraction, due to its relatively long circulatory half-life, may represent a physiologically important circulatory storage form of LH. It would be very interesting to determine if the clearance rates and renal extraction of the dimer forms of some teleost GtH's, which are known to be biologically active (Ng and Idler, 1978a,b), are different from that of the monomer GtH. Also, it would be interesting to determine the clearance rate of GtH in fish with altered renal function to better ascertain the possible role of the kidney in the regulation of circulating GtH levels.

Tissues of the sexually regressed female goldfish, other than the kidney, that accumulate ^{125}I-cGtH greater than the body as a whole include the spleen, hypothalamus and gills (Table 9). However, Table 10 illustrates that when total uptake by the muscle mass is accounted for, this tissue is seen to be a major site of localization. Whether muscle plays a role in degradation of the hormone is not known. The comparison between male and female goldfish did not reveal major sex differences in the tissue distribution of glycoprotein ^{125}I-cGtH when expressed as either uptake per unit weight of tissue or per total organ weight (Tables 9 and 10). The finding that the hypothalamus incorporates more ^{125}I-cGtH than the rest of the brain is interesting in view of the role of the hypothalamus in the regulation of GtH secretion

(Peter *et al.*, 1978; Peter and Crim, 1979). However, similar results were not obtained with sexually maturing fish where the ^{125}I-cGtH uptake of the hypothalamus did not differ from the rest of the brain (data not shown). Further, none of the radioactivity incorporated by the hypothalamus was displaceable with excess unlabelled cGtH in sexually maturing female goldfish. The reason for the discrepancy in ^{125}I-cGtH uptake by the hypothalamus between the regressed and maturing female goldfish is not known.

The values of $t_{\frac{1}{2}_i}$ for cGtH determined in the present study are very different from the published values for a number of mammalian gonadotropins (e.g. see Tables 11, 12 and 13). The largest calculated value of $t_{\frac{1}{2}_i}$ for cGtH determined from sexually regressed female goldfish maintained at 12 ± 1° C (ca. 13 minutes) is considerably less than the widely quoted $t_{\frac{1}{2}_i}$ value of 30 minutes for LH in the rat (Bogdanove and Gay, 1969). The goldfish cGtH $t_{\frac{1}{2}_i}$ values are also less than the most recent estimates 15 to 20 minutes) of LH $t_{\frac{1}{2}_i}$ in the rat (Weick, 1977; Campbell *et al.*, 1978a,b). However, the plasma primary MCR for LH in the rat (8 to 11 $ml \cdot h^{-1} \cdot 100g^{-1}$;Weick,1977; Campbell *et al.*, 1978a,b) is similar to that determined for cGtH in the goldfish, although the MCR of LH in human subjects is substantially less than in the goldfish (0.01 $ml \cdot h^{-1} \cdot 25g^{-1}$) (Kohler *et al.*, 1968). A number of studies have demonstrated that FSH and HCG are cleared much more slowly than LH in a variety of mammalian species in terms of both an increased $t_{\frac{1}{2}_i}$ and a decreased MCR (Bogdanove *et al.*, 1975; Rizkallah *et al.*, 1969; see Tables 11, 12 and 13), suggesting that the clearance of

TABLE 11. Summary of the literature[a] describing the sialic acid content and biological half-life of luteinizing hormone (LH).

Species	Sialic Acid Content (%)	Author	Initial Half-Disappearance[b] Time (Minutes)	Author
human	3.2	Suttajit *et al.* (1971)	60	Coble *et al.* (1969)[c]
	2.8	Shownkeen *et al.* (1973)	16	Kohler *et al.* (1968)[c]
	2.1	Hartree (1971)		
ovine	0.3	Parlow (1968)	28	Geshwind and Dewey (1968)
	0.3	Papkoff *et al.* (1965)	28	Kaltenbach (1974)
			43	Akbar *et al.* (1974)
rat			5	Ascoli *et al.* (1975)
			15	Campbell *et al.* (1978a)
			18	Weick (1976)

a This table is not intended to be a complete compilation; the data shown is representative of that reported from a number of laboratories.

b All values of biological half-life determined from multiexponential decay curves represent the initial rapid clearance of a two-compartment analysis (see METHODS AND MATERIALS).

c Initial biological half-life calculated from their clearance data according to the methods described in APPENDIX I and METHODS AND MATERIALS.

TABLE 12. Summary of the literature[a] describing the sialic acid content and biological half-life of follicle stimulating hormone (FSH).

Species	Sialic Acid Content (%)	Author	Initial Half-Disappearance[b] Time Minutes	Author
human	5.2	Barr and Collee (1967)	180	Coble *et al.* (1969)[c]
	5.0	Barker *et al.* (1969)		
	2.3	Suttajit *et al.* (1971)		
ovine	3.4	Suttajit *et al.* (1971)	102	Akbar *et al.* (1974)
	6	Sherwood *et al* (1970)	120	Kaltenbach *et al.*, unpublished results cited in Akbar *et al.* (1974)
porcine	6	Whitely *et al.* (1978)		
rat			149	Bogdanove and Gay (1969)
			20	Bogdanove *et al.* (1975)

a This table is not intended to be a complete compilation; the data shown is representative of that reported from a number of laboratories.

b All values of biological half-life determined from multiexponential decay curves represent the initial rapid clearance of a two-compartment analysis (see METHODS AND MATERIALS).

c Initial biological half-life calculated from their clearance data according to the methods described in APPENDIX I and METHODS AND MATERIALS.

TABLE 13. Summary of the literature[a] describing the sialic acid content and biological half-life of some selected protein hormones.

Hormone	Species	Sialic Acid Content (%)	Author	Initial Half-Disappearance[b] Time (minutes)	Author
PMSG[c]	equine	10.2	Papkoff (1971) cited in Canfield *et al.* (1971)	8640	Cole *et al.* (1967)
HCG	human	8	Bahl (1969)	320	Rizkallah *et al.* (1969)
		9	Canfield *et al.* (1971)	318	Parlow and Ward (1961)
TSH	human	1.82	Shome *et al.* (1968)	54	Odell *et al.* (1967)
GH	rat			10	Frohman and Bernardis, 1970[d]
	porcine			9	Althen and Gerrits, 1976
PRL	rat			5	Koch *et al.* (1974)
Insulin	porcine			6	Stoll *et al.* (1971)
ACTH	ovine			1	Jones *et al.* (1975)

a This table is not intended to be a complete compilation; the data shown is representative of the reported from a number of laboratories.

b All values of biological half-life determined from multiexponential decay curves represent the initial rapid clearance of a two-compartment analysis (see METHODS AND MATERIALS).

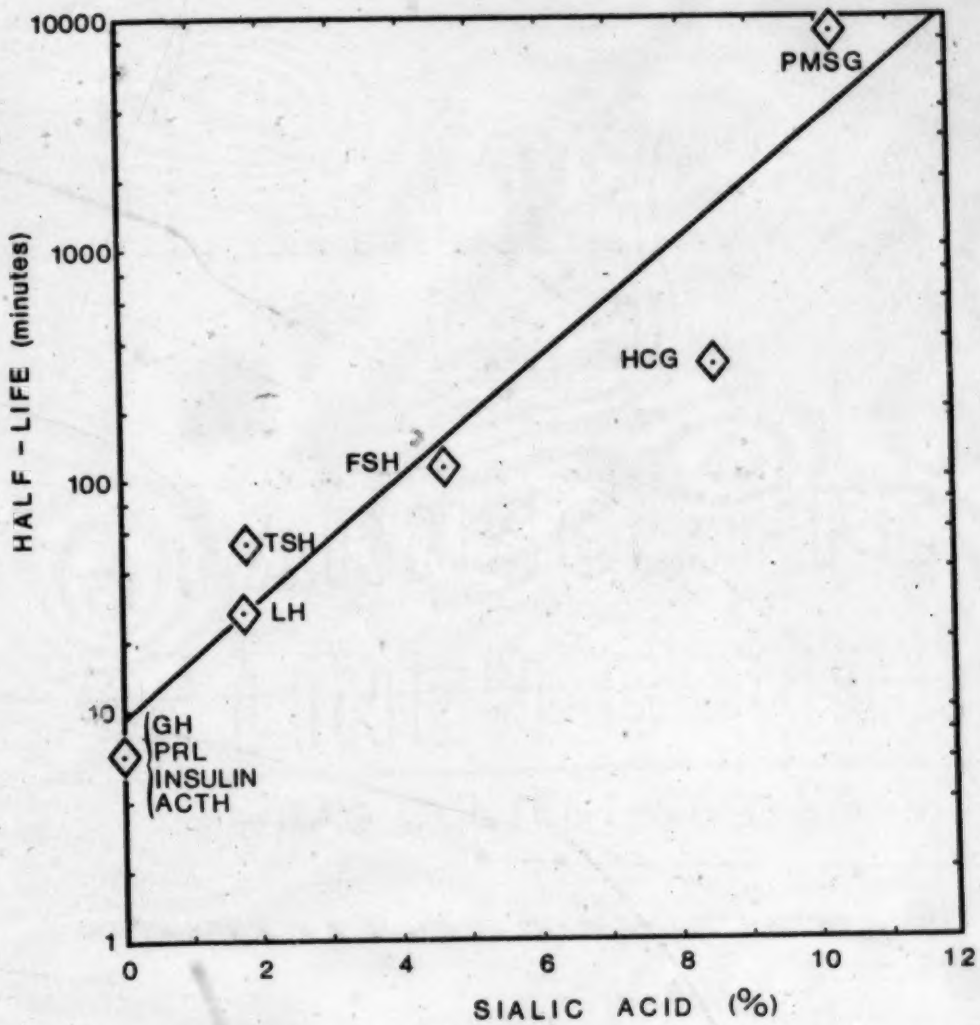
c Abbreviations: PMSG, pregnant mare serum gonadotropin; HCG, human chorionic gonadotropin; TSH, thyroid stimulating hormone; GH, growth hormone; ACTH, adrenocorticotropic hormone.

d Initial biological half-life calculated from their clearance data according to the methods described in APPENDIX I and METHODS AND MATERIALS.

cGtH in the goldfish more closely resembles that of LH than other mammalian gonadotropins. Since the smaller calculated values of $t_{\frac{1}{2}i}$ for cGtH in the goldfish compared to those for LH or FSH in mammalian species are not likely to be attributed to a difference in distribution volumes (see above), it is possible they reflect major differences in the animals metabolic clearance mechanisms. Several possibilities may explain why the calculated value of $t_{\frac{1}{2}i}$ for ^{125}I-cGtH in the goldfish are less than the corresponding values for mammalian gonadotropins.

Firstly, it is possible the decreased values of $t_{\frac{1}{2}i}$ for goldfish GtH compared to those determined for mammalian GtH's may be related to the tissue distributions of the GtH's. In the goldfish, the kidney is clearly the most active tissue in terms of ^{125}I-cGtH uptake, in contrast to gonads, liver and other tissues (Table 9). Although the *in vivo* pattern of ovarian uptake of radioiodinated human chorionic gonadotropin and radioiodinated luteinizing hormone in mice (Lunenfeld and Eshkol, 1967; Kammerman and Canfield, 1972; Canfield *et al.*, 1971) and rats (Tsuruhara *et al.*, 1972b) are similar to that observed in the goldfish (Figure 6), the relative contribution of the gonad compared to other tissues, such as liver and kidney, appears to be much greater in mammals (e.g. see Kammerman and Canfield, 1972) than in the goldfish. It is possible that an increased rate of irreversible blood clearance of GtH by the kidney is, in part, responsible for the faster $t_{\frac{1}{2}i}$ in goldfish. It is of note that a number of recent studies have emphasized a more important role of the kidney in the regulation of

Fig. 19. A semi-logarithmic plot of initial biological half-life ($t_{\frac{1}{2}_i}$) versus per cent sialic acid content. The data are mean values calculated from Tables 11, 12 and 13. Values of $t_{\frac{1}{2}_i}$ are included only from studies employing a hormone homologous with the species in which the plasma clearance was determined. The values are based upon representative data reported from a number of laboratories and do not represent a complete compilation.


10000
1000
100
10
1
HALF - LIFE (minutes)
PMSG
HCG
FSH
TSH
LH
GH
PRL
INSULIN
ACTH
0
2
4
6
8
10
12
SIALIC ACID (%)

mammalian gonadotropin clearance (Ascoli and Puett, 1976a; de Kretser, 1973; Ascoli *et al.*, 1975; Lee and Ryan, 1974) than previously supposed (Morell *et al.*, 1971; Kammerman and Canfield, 1972). However, the similarity between the calculated values of overall and primary plasma MCR GtH in the goldfish and mammals suggests that other processes may also contribute to the observed rapid goldfish $t_{\frac{1}{2}i}$.

An alternative hypothesis explaining the shorter initial $t_{\frac{1}{2}i}$ values in the goldfish concerns differences in the chemistry of cGtH compared to mammalian gonadotropins. The sialic acid content of HCG is substantially greater than that of FSH, LH and the teleost glycoprotein gonadotropins (Tables 11, 12, 13 and 14). Since the published values of $t_{\frac{1}{2}i}$ for HCG (Canfield *et al.*, 1971) are much greater than other gonadotropins (Table 11, 12 and 14) and since progressive desialylation of HCG (Van Hall, 1971b) and FSH (Morell *et al.*, 1971) results in an increased rate of plasma clearance and concomitant accumulation in the liver (Morell *et al.*, 1971), it is apparent that the sialic acid content influences the rate of metabolism of these hormones *in vivo*. In support of this, a survey of the literature suggests a strong correlation between the content of sialic acid and the $t_{\frac{1}{2}i}$ of a number of mammalian gonadotropins (Figure 19). The sialic acid content of teleost glycoprotein GtH's (Table 14), with the exception of plaice GtH (D.R. Idler, personal communication), is considerably less than that of most mammalian gonadotropins (Tables 11, 12 and 13). If the content of sialic acid also influences the $t_{\frac{1}{2}i}$ of teleost GtH's, this may be an explanation for the shorter $t_{\frac{1}{2}i}$ found here for goldfish.

TABLE 14. Summary of some recent information describing the sialic acid content and initial biological half-life of some teleost glycoprotein gonadotropins.

Species	Sialic Acid Content (%)	Author	Initial Half-Disappearance Time (minutes) [a]	Author
carp	0.35	Burzawa-Gerard (1969)	4 - 13	present study
	0	Jollès *et al.* (1977)		
	0.85	D.R. Idler (pers. comm.)		
salmon	1.5	D.R. Idler (pers. comm.)		
plaice	3.5	D.R. Idler (pers. comm.)		

a Initial biological half-life calculated as described in METHODS AND MATERIALS.

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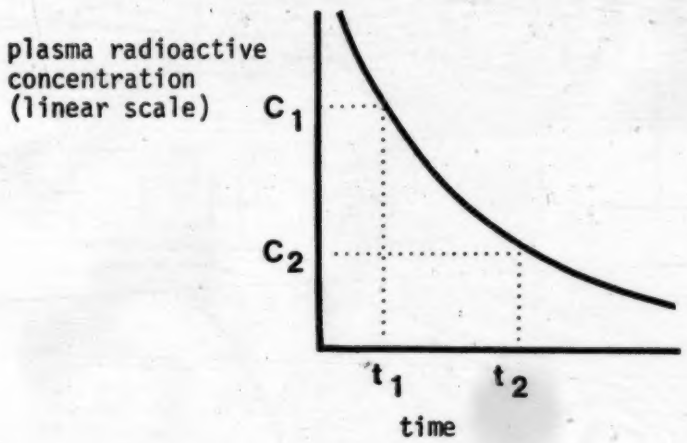
APPENDIX I

Derivation of the biological half-life ($t_{\frac{1}{2}}$)

If the clearance of ^{125}I-cGtH from the plasma of goldfish depended only upon first order kinetics, a plot of per cent of injected dose per ml plasma against t on linear coordinates would produce a curve similar to that shown in Figure 1.

Figure 1:



The resulting curve may be described by the equation:

$$x'(t) = A\, e^{-\alpha t} \qquad (1)$$

Taking the natural logarithm of both sides of (1) yields:

$$\ln x'(t) = \ln A - \alpha t \qquad (2)$$

Thus when x'(t) is plotted on semi-logarithmic graph paper a straight line of slope α and ordinate intercept ln A is obtained (Figure 2)

and

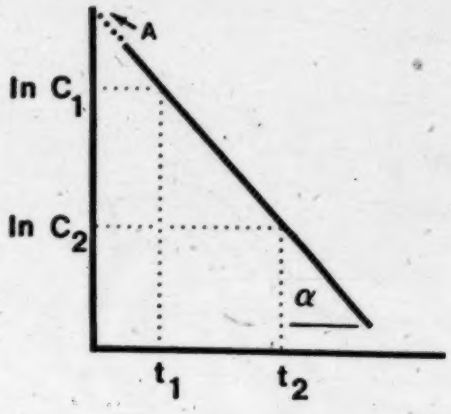


Figure 2.

$$\alpha = \frac{\ln C_1 - \ln C_2}{t_1 - t_2} \qquad (3)$$

The biological half-life is defined as $t_1 - t_2$ when $C_2 = \frac{C_1}{2}$ and (3) becomes:

$$\alpha = \frac{\ln C_1 - \ln \left(\frac{C_1}{2}\right)}{t_{\frac{1}{2}}} \qquad (4)$$

$$\alpha = \frac{\ln C_1 - (\ln C_1 - \ln 2)}{t_{\frac{1}{2}}}$$

$$\alpha = \frac{\ln 2}{t_{\frac{1}{2}}}$$

Therefore $t_{\frac{1}{2}} = \frac{0.693}{\alpha}$

The extension to a two compartment or higher exponent system proceeds in an identical manner.

APPENDIX II

Metabolic Clearance Rate

Metabolic clearance rate (MCR) is defined as the volume of blood or plasma that is completely and irreversibly cleared of hormone per unit time (Tait and Burstein, 1964). In the short time interval dt, a volume of plasma is completely and irreversibly cleared of ^{125}I-cGtH, and if x'(t) is the radioactive concentration at time t, the amount cleared $dR = MCR \cdot x'(t)\, dt$ (1)

If the total amount cleared is R, then

$$R = \int_0^\infty MCR \cdot x'(t)\, dt, \tag{2}$$

and since the total amount cleared must equal the total amount injected

$$\text{TOTAL AMOUNT INJECTED} = \int_0^\infty MCR \cdot x'(t)\, dt \tag{3}$$

All radioactive concentrations may be measured as a per cent of injected dose; the total amount injected being 1 or 100 per cent, enabling (3) to be rewritten as

$$1 = \int_0^\infty MCR \cdot x'(t)\, dt \tag{4}$$

If MCR is constant i.e. assuming steady-state conditions (Atkins, 1969)

$$1 = MCR \int_0^\infty x'(t)\, dt \tag{5}$$

$$\text{or} \qquad MCR = \frac{1}{\int_0^\infty x'(t)\, dt} \tag{6}$$

MCR is, then, the reciprocal of the area beneath the MCP. This is the most general method of estimating MCR which is independent of the number of compartments or model used and assumes only steady-state conditions (Gurpide *et al.*, 1964).

Although the derivation of (6) is straightforward, its evaluation is not. Tait and Burstein (1964) assuming a two-pool open system, compute (6) in the following manner:

$$MCR = \frac{1}{\int_0^\infty x'(t)\,dt} \quad \text{and since } x'(t) = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

$$MCR = \frac{1}{\int_0^\infty (A\,e^{-\alpha t} + B\,e^{-\beta t})\,dt}$$

Using methods of integral calculus:

$$MCR = \frac{1}{\int_0^\infty A\,e^{-\alpha t}\,dt + \int_0^\infty B\,e^{-\alpha t}\,dt}$$

$$MCR = \frac{1}{A\int_0^\infty e^{-\alpha t}\,dt + B\int_0^\infty e^{-\alpha t}\,dt}$$

$$MCR = \frac{1}{A\left(-\frac{e^{-\alpha t}}{\alpha}\Big|_0^\infty\right) + B\left(-\frac{e^{-\beta t}}{\beta}\Big|_0^\infty\right)}$$

$$MCR = \frac{1}{A\left(-\frac{e^{-\alpha\infty}}{\alpha} + \frac{e^{-\alpha 0}}{\alpha}\right) + B\left(-\frac{e^{-\beta\infty}}{\beta} + \frac{e^{-\beta 0}}{\beta}\right)}$$

$$MCR = \frac{1}{A\left(\frac{1}{\alpha}\right) + B\left(\frac{1}{\beta}\right)}$$

$$MCR = \frac{\alpha\,\beta}{A\,\beta + B\,\alpha} \qquad (7)$$

TABLE 15. Comparison of five methods to compute the metabolic clearance rate (MCR) of ^{125}I-cGtH in sexually regressed female goldfish (GSI = 1.70 ± 0.35%) at 12 ± 1° C, using the single injection technique.

radioactivity analysed	MCR (ml · h^{-1} · $25g^{-1}$)				
	primary	overall			
	$\alpha \cdot A^{-1}$	$(\int_5^{120} x\,(t))^{-1}$ analytically	$(\int_5^{120} x\,(t))^{-1}$ numerically	$(\int_0^{120} x\,(t))^{-1}$ analytically	$(\int_0^{\infty} x\,(t))^{-1}$ analytically
Total	10.44 ± 0.32[a]	2.23 ± 0.07	2.54 ± 0.05	1.79 ± 0.06	1.45 ± 0.05
TCA-precipitated	10.41 ± 0.34	2.27 ± 0.09	2.27 ± 0.03	1.85 ± 0.07	1.49 ± 0.06
Immunoprecipitated	10.88 ± 0.32	2.40 ± 0.07	2.39 ± 0.03	1.93 ± 0.05	1.66 ± 0.05

a All data are mean ± SE.

Although this estimate of MCR is utilized by a number of experimenters (Higgs and Eales, 1976; Donaldson and Fagerlund, 1968, 1969, 1970) it is readily apparent that errors in the estimation of the curve parameters are reflected and may be magnified in the determination of MCR. Other workers have evaluated (6) from $t = 0$ or $t = 5$ to $t = 120$ or some other finite time interval, attempting to minimize the extrapolation of the disappearance profile beyond the experimental data (Campbell *et al.*, 1978a). Such a procedure will give a smaller estimate of the area and correspondingly larger estimate of MCR (Table 15). Normand and Fortier (1970) employ a numerical technique to integrate the area beneath the MCP. Their application of the trapezoidal rule demonstrates its computational simplicity, statistical advantage and elimination of any need for characterization of a model. However, unless sampling is begun immediately after injection and continued until the plasma specific activity is negligible compared to injected dose, this method will also underestimate total area and correspondingly overestimate MCR (Table 15).

Considering only the fast initial disappearance of tracer from the first pool of a multicompartmental system, Campbell *et al.* (1978a) have defined a primary metabolic clearance rate equal to $\alpha \cdot A^{-1}$. By ignoring a large percentage of the total area beneath each MCP the primary MCR will be much greater than the overall MCR (Table 15). Implicit in this computation of primary MCR is that the rapid clearance of labelled hormone from the initial vascular compartment follows single order kinetics.

APPENDIX III

For each experiment, consisting of 5 individual samples at each of 7 sampling times, a single value for MCR, $t_{\frac{1}{2}}$ and V_i is computed. To facilitate comparison between different experiments, it is essential that a relevant estimate of the error be obtained for each of these values. As it is not possible to repeat individual experiments a large number of times, an estimate of the error must be determined from the 35 samples of each experiment. The general theorem that follows provides a valid method of estimating the error of MCR, $t_{\frac{1}{2}}$ and V_i or any function of a number of values each with known estimate of error.

$$\text{Let } N = f(u_1, u_2, \ldots\ldots, u_n) \tag{1}$$

denote any function of the values $u_1, u_2, \ldots\ldots u_n$ which are subject to the random errors $e_{u_1}, e_{u_2}, \ldots\ldots e_{u_n}$, respectively. These errors in the u_i's will cause an error e_N in the function N according to:

$$N + e_N = f(u_1 + e_1, u_2 + e_2, \ldots\ldots, u_n + e_n) \tag{2}$$

The right hand side of (2) can be expanded by Taylor's theorem for a function of several variables (Lang, 1968).

$$N + e_N = f(u_1, u_2, \ldots\ldots, u_n) + \sum_{i=1}^{n} \frac{\partial f}{\partial u_i} e_i$$

$$+ \tfrac{1}{2} \left(e_i^2 \frac{\partial^2 f}{\partial u_i^2} + \ldots\ldots \frac{\partial^2 f}{\partial u_n^2} (e_n)^2 \right)$$

$$+ 2e_1 e_2 \frac{\partial^2 f}{\partial u_1 \partial u_2} + \ldots\ldots \quad + \ldots\ldots \qquad (3)$$

If the errors e_i are relatively small, the products, squares and higher powers may be neglected; and (3) may be rewritten as:

$$N + e_N = f(u_1, u_2, \ldots\ldots, u_n) + \sum_{i=1}^{n} \frac{\partial f}{\partial u_i} e_i \qquad (4)$$

The variance of (4) is:

$$\text{VAR}\ (N + e_N) = \text{VAR}\ f(u_1, u_2, \ldots\ldots, u_n) + \text{VAR} \left(\sum_{i=1}^{n} \frac{\partial f}{\partial u_i} e_i \right). \qquad (5)$$

Since the u_i's are constants $\text{VAR}\ (f(u_1, u_2, \ldots\ldots, u_n)) = 0$

and (5) becomes:

$$\text{VAR}\ (N + e_N) = \text{VAR} \sum_{i=1}^{n} \frac{\partial f}{\partial u_i} e_i \ . \qquad (6)$$

Expansion of the right-hand side of (6) yields

$$= \sum_{i=1}^{n} \left(\frac{\partial f}{\partial u_i} \right)^2 \left(s_i \right)^2 + 2 \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \frac{\partial f}{\partial u_i} \frac{\partial f}{\partial u_j} \text{CoV}\ (e_i, e_j) \qquad (7)$$

(Hogg and Craig, 1970).

Since many non-linear least squares curve fitting programs

provide a correlation matrix (ρ_{ij}) and assymptotic standard deviations ($s_{e_{ii}}$) of the converged parameters, it is convenient to write (7) as:

$$= \sum_{i=1}^{n} \left(\frac{\partial f}{\partial u_i}\right)^2 \cdot \left(s_{e_i}\right)^2 + 2 \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \frac{\partial f}{\partial u_i} \frac{\partial f}{\partial u_j} \rho_{ij} s_i s_j \qquad (8)$$

or in matrix notation where

$\underline{V}$ is an n x 1 vector $= \begin{bmatrix} \frac{\partial f}{\partial u_1} \cdot s_1 \\ \vdots \\ \frac{\partial f}{\partial u_n} \cdot \bar{s}_n \end{bmatrix}$

and ρ is an n x n matrix = (ρ_{ij}) as:

$$\text{VAR}\ (N + e_N) = \underline{V}^1 \cdot \rho \cdot \underline{V} \qquad (9)$$

The application of the general formula is illustrated by example. The distribution space of the first compartment (V_i) and the estimate of error (s_{V_i}) is determined using the data obtained from the MCP tracing the disappearance of immunoprecipitated ^{125}I-cGtH in sexually regressed female goldfish, *Carassius auratus*, maintained at 12 ± 1° C.

TABLE 16. Immunoprecipitated plasma radioactivity from regressed female goldfish (GSI = 1.70 ± 0.35%) at T = 12 ± 1° C: a part of the computerized output from a non-linear least squares program BMD 07R (Dixon, 1974).

Parameter	Value	Standard Deviation	Correlation Matrix	
	ml	ml	A	B
A	1.266	0.492	1.000	0.571
B	0.426	0.0822	0.571	1.000

(i) evaluation of V_i: $V_i = (A+B)^{-1} = 0.591$ ml

(ii) evaluation of the variance of V_i, using equation (9),

$$VAR(V_i) = (-(A+B)^{-2}\cdot s_A,\ -(A+B)^{-2}\cdot s_B) \begin{pmatrix} 1.000 & 0.571 \\ 0.571 & 1.000 \end{pmatrix} \begin{pmatrix} -(A+B)^{-2}\cdot s_A \\ -(A+B)^{-2}\cdot s_B \end{pmatrix}$$

$= 0.0336\ (ml)^2$. Therefore s_{V_i} 0.183 ml and SEM 0.031 ml plasma.

APPENDIX IV

Ovulatory Surge of Gonadotropin in the Goldfish, *Carassius auratus*

N.E. Stacey

A.F. Cook

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Running head: Goldfish Ovulatory GtH Surge

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ABSTRACT

The periovulatory pattern of circulating gonadotropin is described for the first time in a poikilothermic vertebrate. In sexually mature female goldfish (Carassius auratus) maintained on a 16L:8D photoperiod, ovulation occurs during the latter part of the dark phase, approximately 20 h after the fish are warmed from 13 to 21°C. Serum gonadotropin levels increase gradually during the first half of the light phase, and by the latter part begin to increase rapidly, reaching the peak of the gonadotropin surge by the onset of the dark phase. Gonadotropin levels remain high through the period of ovulation, and decrease markedly by the onset of the following light phase. The findings indicate that the goldfish provides a valuable model for the study of ovulation in teleosts.

INTRODUCTION

In many mammals (Wildt et al., 1978) and in the domestic fowl (Furr et al., 1973) ovulation is preceded by a rapid and transient increase (surge) in plasma gonadotropin (GTH). Although a role of GtH in teleost ovulation has been clearly demonstrated (Chaudhuri, 1976), there is no direct evidence for a preovulatory surge of GtH in teleosts. Plasma GtH levels in several salmonid species are elevated prior to and following ovulation (Crim et al., 1973, 1975); however, the time course of these changes has not been described. Breton et al. (1972) reported elevated GtH levels in goldfish (Carassius auratus) that had ovulated, but did not detect increased levels prior to ovulation. We report here the presence of a preovulatory surge of GtH in the female goldfish.

METHODS

Female goldfish of the common or comet variety (20-40 g body weight) were purchased from Grassyfork Fisheries Co., Inc., Martinsville, Indiana, from February to April, and kept in 1500 l flow-through stock aquaria (13±1°C;16L:8D, lights on at 0400 h) for at least one month before use. Ewos pellets were fed ad libitum at least twice per day.

Ovulation was induced by an increase in termperature as described by Yamamoto et al. (1966); with this technique, a proportion of sexually mature female goldfish will ovulate within 36 h of a temperature increase from 13 to 21°C. At 1800 h on day 1, fish with mature ovaries (indicated by a soft, distended abdomen) were removed from stock aquaria, fin-clipped for individual identification, and transferred to 45 l standing-water ovulation aquaria (5-7 fish per aquarium) which warmed from 13±1°C to 21±1°C over a period of 10 h (Fig. 1). Ovulation aquaria were provided with coarse sand over a sub-sand filter, a charcoal and glass wool box filter, and sufficient floating artificial plants (each made from 100 ten-cm strands of green acrylic fiber) to cover at least 50% of the water surface. Between 0900 and 1100 h on day 2, one or two spermiating male goldfish and a female which had been induced to perform spawning behaviour by injection of prostaglandin $F_{2\alpha}$ (Stacey, 1976) were added to each ovulation aquaria; Yamazaki (1965) states that in warm water the presence of sexually active males and aquatic plants stimulates ovulation. The spermiated males and prostaglandin-injected females were removed near the end of the light period on day 2. All experimental

fish were fed a commercial dry food (Tetramin) at random times between 0400 and 1800 h on day 2.

One, two, or three blood samples were taken from each fish at various times from 1800 h on day 1, at the time of removal from stock aquaria, to 1600 h on day 3 (Fig. 1). Most fish were sampled twice, at least four h apart. Prior to blood sampling, fish were anaesthetized in MS222 (tricaine methanesulphonate; 0.05% solution). Blood samples, about 150 µl preovulatory and 300 µl postovulatory, were taken from the caudal vasculature with a 1 ml syringe fitted with a 25 gauge needle, allowed to clot on chipped ice for 1-2 h, and centrifuged at 760 X g (4°C) for 25 min. To prevent bacterial growth, 1 µl of thiomerosolate (1% solution) was added to each serum sample prior to freezing on dry ice and storage at -30°C. Serum samples were analyzed by a radioimmunoassay (RIA) for GtH employing antibodies to carp GtH, as described previously (Crim et al., 1976; Hontela and Peter, 1978).

Ovulation was indicated by release of a stream of oocytes from the ovipore following application of gentle pressure to the abdomen. Fish were checked for ovulation hourly from 2300 h on day 2 until 0400 h on day 3; fish which had failed to ovulate in this interval were also checked several more times during the light phase on day 3. A dim red light (4.3 lux) was used to locate fish in ovulation aquaria during the dark phase. When blood samples were taken during the dark phase, fish were anaesthetized in the dark, sampled in light, and returned to dark prior to full recovery from anaesthesia.

RESULTS

Under the temperature and photoperiod conditions used in this study, ovulation occurs between 0000 and 0400 h on day 3 (Fig. 1). The blood sampling did not affect the time of ovulation, the magnitude of the GtH surge, or the levels of serum GtH in subsequent samples. Thus, the mean GtH values and times of ovulation in Fig. 1 represent groups comprised of fish sampled once, twice, or three times. Fish which were warmed and placed in ovulation aquaria but did not ovulate (non-ovulatory fish) showed a significant increase ($p<0.05$ in serum GtH levels between 0800 and 1200 h on day 2, coincident with increasing water temperature; however, there was no significant change in the GtH levels of these non-ovulatory fish throughout the remainder of the 44 h sampling period. Ovulatory and non-ovulatory fish had similar GtH levels at 1800 h on day 1, immediately prior to transfer to ovulation aquaria. By 0800 the following morning, the GtH levels in ovulatory fish were significantly greater ($p<0.001$) than the levels in the non-ovulatory group. In ovulatory fish serum GtH levels increased further at 1200 h on day 2, and by 1600 h were significantly greater than either the 0800 or 1200 h mean levels (Fig. 1). Ovulatory fish sampled at the end of the light period on day 2 (2000 h) all had serum GtH levels greater than 100 ng/ml and reached a maximum with a mean of 168 ± 14 ng/ml at 0000-0400 h on day 3 during the period of ovulation. Serum GtH levels decreased rapidly in ovulated fish, reaching presurge levels by 0800 h on day 3; a similar decline occurs following clomiphene-induced GtH surges in male and female carp (Breton et al., 1975).

DISCUSSION

The present report provides the first demonstration of a preovulatory surge of GtH in a poikilothermic vertebrate. The results of this study support the finding of Yamamoto and Yamazaki (1967) that vacuolization of pituitary basophils is maximal approximately 10 h prior to follicular rupture in female goldfish. The 8-12 h interval between the intial sharp rise in plasma GtH (1600 h, day 2) and the time of spontaneous ovulation in this study (0000-0400 h, day 3) agrees well with the latency to induced ovulation following injection of HCG (unpub. results). Our data also condirm and extend those of Breton et al. (1972) who reported elevated mean serum GtH levels (approx. 50 ng/ml) in female goldfish before noon on the day of ovulation (day 3 in this study).

Elevated serum GtH levels also have been reported in preovulatory and ovulated pink salmon, Onocorhynchus gorbuscha (Crim et al., 1973), brook trout, Salvelinus fontinalis, and sockeye salmon, O. nerka (Crim et al., 1975); unfortunately, these studies provided no information as to the time-course of the preovulatory increase in serum GtH or the latency between the GtH increase and ovulation. In contrast to the situation in goldfish, post-ovulatory GtH levels in several samonid species remain high for at least several days after ovulation (O. nerka, Crim et al., 1975; salmo gairdnerii, Fostier et al., 1978).

The manner in which temperature increase, aquatic vegetation and male courtship might facilitate and synchronize spontaneous ovulation

is not clear. Preliminary results suggest aquatic vegetation is the most important stimulus for ovulation and that male courtship has no effect. Temperature increase may act simply by raising basal GtH levels, an effect seen in both non-ovulatory (Hontela and Peter, 1978) and ovulatory fish. As fish warmed at 1200 h on day 1 may ovulate at 0000-0400 h on day 2 or succeeding days, but not at other times (unpub. results), photoperiod, and not temperature, appears responsible for the synchrony in ovulation times observed in this study.

Breton et al. (1972) and Hontela and Peter (1978) have described daily variations of serum GtH in mature female goldfish. The highest level occurred about 4 to 6 h after the onset of light in fish kept at warm temperature under long photoperiod. As the time of the maximum daily GtH levels in these studies does not coincide with the time of the preovulatory surge described in this report, it is possible that in the goldfish, as has been suggested in mammals (Flerko, 1970), separate mechanisms may regulate tonic and ovulatory GtH secretion.

In summary, at 21°C and 16L:8D, the preovulatory surge of GtH in the goldfish appears to result from an extended (ca. 8 h) period of increased GtH secretion which begins in the last four hours of the light phase and induces ovulation in the latter half of the dark phase. The predictability of this response and the ease with which the time of ovulation can be determined indicate that the goldfish will prove a useful tool in the study of endocrine events associated with spontaneous ovulation.

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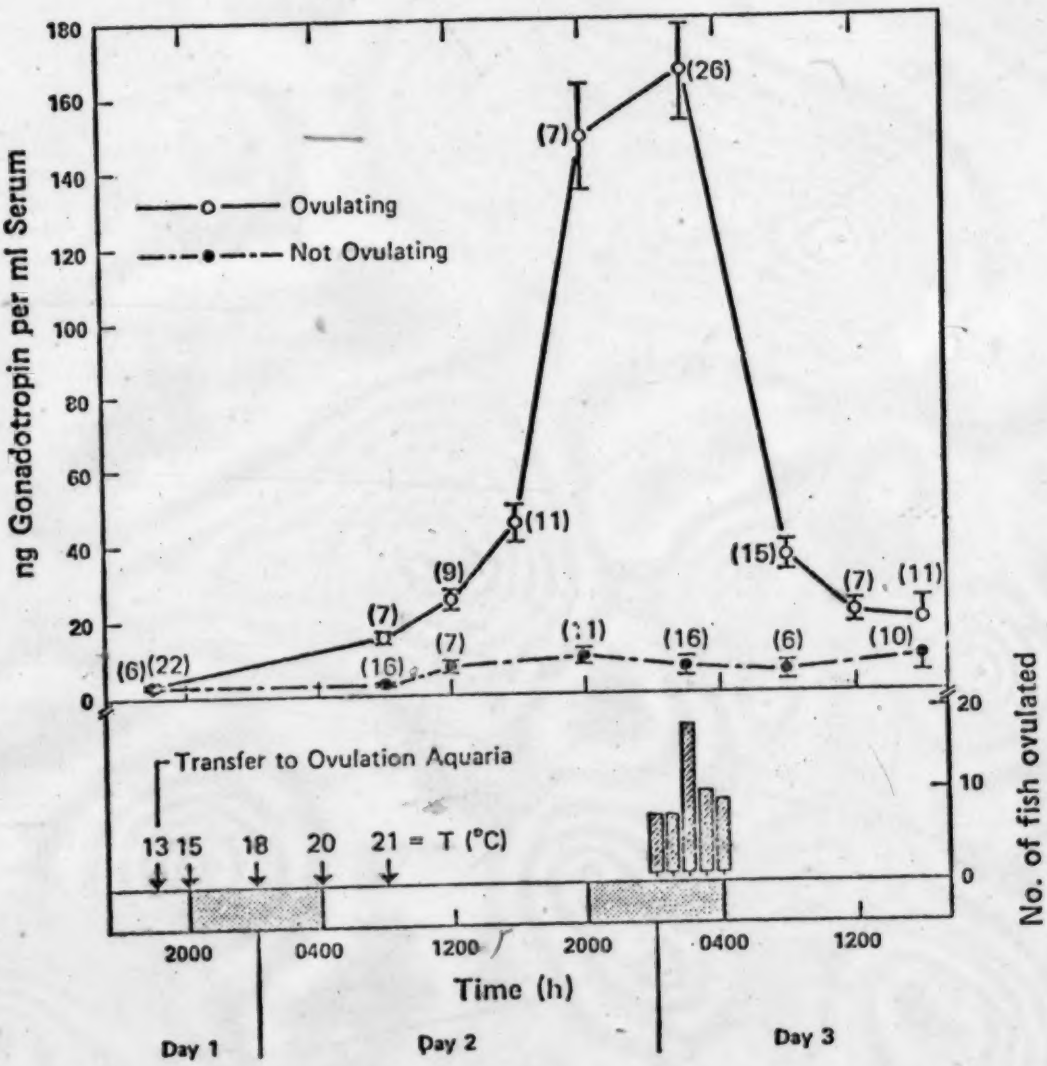
FIGURE 1. TEXT

Times of ovulation and serum gonadotropin (GtH; mean ± SEM) profiles of sexually mature female goldfish kept on a 16L:8D photoperiod and warmed from 13±1°C to 21±1°C. The number of fish ovulating each hour is shown in the lower graph. The GtH levels measured in serum from ovulatory fish sampled at the time of ovulation (0000-0400 h on day 3) were pooled and expressed as a mean value at 0200 h on day 3. Differences between means within the ovulatory and non-ovulatory groups were determined by ANOVA and Duncan's Multiple Range Test (Steele and Torrie, 1960) using log transformed data. The unpaired Student's T-test for groups with dissimilar variances (Steele and Torrie, 1960) was used to compare values at each sample time.


ng Gonadotropin per ml Serum
180
160
140
120
100
80
60
40
20
0
Ovulating
Not Ovulating
(6)
(22)
(16)
(7)
(9)
(7)
(11)
(7)
(26)
(7)
(11)
(16)
(6)
(15)
(7)
(11)
(10)
Transfer to Ovulation Aquaria
13 15 18 20 21 = T (°C)
20
10
0
No. of fish ovulated
2000
0400
1200
2000
0400
1200
Time (h)
Day 1
Day 2
Day 3